

INVESTIGATIONS IN FISH CONTROL

80. Effects of Antimycin A and Rotenone
on Macrobenthos in Ponds
81. Aquatic Macroinvertebrates in a Small Wisconsin
Trout Stream Before, During, and Two Years After
Treatment with the Fish Toxicant Antimycin



UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

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(Reports 41 through 43 are in one cover.)

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57. Acute Toxicities of 3-Trifluoromethyl-4-nitrophenol (TFM) and 2',5-Dichloro-4'-nitro-salicylanilide (Bayer 73) to Larvae of the Midge *Chironomus tentans*, by J. A. Kawatski, M. M. Ledvina, and C. R. Hansen, 1975. 7 pp.
58. Acute Toxicity of the Lampricide 3-Trifluoromethyl-4-nitrophenol (TFM) to Nymphs of Mayflies (*Hexagenia* sp.), by C. R. Fremling. 1975. 8 pp.
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Effects of Antimycin A and Rotenone on Macrobenthos in Ponds

by

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Abstract

Samples of macrobenthos, collected over a 14-month period from nine 0.03-ha experimental ponds at the Fish-Pesticide Research Laboratory, Columbia, Missouri, were analyzed to determine the long- and short-term effects of antimycin A and rotenone. The ponds were characterized by an abundance of bushy pondweed (*Najas guadalupensis*) and by the absence of fish. Treatment concentrations of 0.5 mg/l of rotenone and 20 $\mu\text{g/l}$ of antimycin and concentrations of 2.0 mg/l of rotenone and 40 $\mu\text{g/l}$ of antimycin were applied. There were no effects on species diversity, emergence, seasonal dynamics, abundance, or relative numbers of taxa that could be attributed to either toxicant. Periods of spring emergence, summer buildup, and fall emergence of insects were closely associated with the seasonal development and decline of vegetation.

The short- and long-term effects on invertebrate macrobenthos of treating bodies of water with rotenone and antimycin A lack adequate documentation. Most benthic organisms appear to be unaffected by either toxicant at concentrations applied for fish eradication (Brown and Ball 1942; Walker et al. 1964; Gilderhus et al. 1969; Schoettger et al. 1967; Smith 1972). However, there are reports of mortality among specific taxa exposed to low concentrations of rotenone (Cushing and Olive 1956; Brown and Ball 1942) and to high concentrations of antimycin A (H. Howell et al. unpublished data; Walker et al. 1964; Lesser 1972).

The purposes of this study were to: (1) identify any short- or long-term changes in species abundance in pond benthos resulting from the application of rotenone or antimycin, (2) determine any difference in abundance of benthos which may result from use of different concentrations of toxicants, (3) determine the time required for affected populations of organisms to recover from rotenone and antimycin treatment, and (4) determine whether emergence of benthic organisms is affected by rotenone or antimycin treatment.

The complete qualitative and quantitative analyses of benthic collections, June-October 1971 and April-August 1972, which are the basis for this paper, are presented in an appendix.

The appendix also provides analysis of the seasonal changes in benthic populations: summer and winter development, fall and spring emergence. This description of benthic community dynamics in these experimental ponds is distinctive because it concerns a habitat which is heavily vegetated and devoid of predatory fish populations. Dense vegetation provides unusual cover, abundant food, and more habitat niches than occur in nonvegetated bottom sediments. Vegetation also has the potential for causing low concentrations of dissolved oxygen, either directly through respiration or indirectly through decomposition. The absence of fish may modify species abundance and composition of the benthos because the populations are not subject to predation by fish.

Methods

Description of Study Area

This study was conducted on nine similar ponds at the Fish-Pesticide Research Laboratory, Columbia, Missouri. Average dimensions of the standing water mass were 21.4 \times 15.6 \times 0.6 m; average surface area was 0.03 ha; and average volume was 251.3 m³. Pond bottoms were sloped; water depths ranged from about 0.3 to 1.2 m. The soil type was Mexico silt loam.

Experimental Design

The nine experimental ponds, A through I, differed in past use and in length of time they had held water. (Correspondence of pond lettering to the Fish-Pesticide Research Laboratory numbering system is: A, 24; B, 16; C, 23; D, 18; E, 20; F, 19; G, 17; H, 22; and I, 21.) Before the study began, all ponds were drained for 2 weeks of drying, as a means of reducing species differences between ponds and establishing similar successional stages. A random treatment design provided two control ponds, three ponds for rotenone treatment, and four ponds for antimycin treatment. Sample sites were randomized as to location and depth in each pond after the pond was divided into 16 3.0- × 4.5-m sampling rectangles.

Sampling Methods

From 1 June 1971 to 31 August 1972, 121 samples were collected and analyzed. Four samples per pond—two from the shallow area and two from the deep area—were collected at monthly intervals for the 14-month period. From 1 June to 31 August each year, collections were made at 14-day intervals. In addition, more intensive sampling preceded and followed treatment: the interval in days was gradually decreased before treatment in the sequence 14, 7, 3, 2, 1, and treatment; and then gradually increased after treatment in the sequence 1, 2, 3, 7, and 14 days. The sampling device was a modified Ekman dredge, 231 cm².

Samples were washed in the field through a screen with 11.8 meshes per linear centimeter. The vegetation, benthic organisms, and debris remaining on the screen were placed in jars containing 10% formalin. In the laboratory the macro-vegetation was removed and examined for benthic organisms, and the remaining detritus with organisms was placed in a saturated aqueous solution of Epsom salts (magnesium sulfate) and stirred so that the organisms floated to the surface. This is a modification of a flotation procedure described by Anderson (1959), in which sugar was replaced with Epsom salts.

Emergence cages—one in each pond—were operated from 7 to 30 April 1972. The wooden and nylon cages, 1.0 m square by 20 cm high, were 1 to 4 m from shore over water about 1 m deep. Adult insects were removed from the covering screens at weekly intervals and preserved in 95% ethanol.

Computation of Species Diversity Index

A species diversity index derived from the informa-

tion theory (Wilhm and Dorris 1968) was estimated by the formula

$$\bar{d} = - \sum (n_i/n) \log_2 (n_i/n)$$

where \bar{d} = estimate of diversity, n_i = number of individuals in the i th taxon, and n = total number of individuals in the sample.

Application of Toxicants

The ponds were treated in late August, near the usual time of pond treatment (September) in the Midwest. Concentrations of rotenone and the initial concentrations of antimycin were those recommended for fish eradication by Kinney (1968) and Lennon and Berger (1970), respectively.

All ponds were treated on the afternoon of 25 August 1971. Sand formulated antimycin (Fintrol 5) was applied to ponds F, G, H, and I. An initial low treatment concentration of 1.5 μ g/l was applied to ponds F and G, and an initial heavy treatment concentration of 10 μ g/l to ponds H and I. Applications of emulsifiable rotenone (Noxfish; 5% active ingredient) were made in ponds C, D, and E. Ponds C and D received a low treatment concentration of 0.5 mg/l and Pond E a heavy treatment of 2.0 mg/l. Ponds A and B were untreated controls.

Although rotenone concentrations selected were adequate, the treatments with antimycin were ineffective in eradicating fish held in cages in the ponds with pH values greater than 9.0 (Table 1). After completing toxicity tests to determine adequate concentrations of antimycin (Table 2), we re-treated the ponds on 1 September 1971 at low concentrations of 20 μ g/l (F and G) or high concentrations of 40 μ g/l (H and I). The low concentration in ponds F and G produced a complete kill of all bluegills (*Lepomis macrochirus*); however, the high concentration in H and I yielded only a partial kill of bluegills 130 to 180 mm long (Table 3). The lessened effect of the 40 μ g/l concentration was possibly related to the higher pH values in ponds H and I. The pH values were 9.0 in pond F and 9.5 in pond G at 1000 h, and 9.6 in pond H and 9.7 in pond I at 1100 h.

Classification

The classification scheme for the Chironomidae follows Sublette and Sublette (1965), except that we assigned generic status to four of the subgenera of Sublette and Sublette: *Dicrotendipes*, *Harnischia*, *Endochironomus*, and *Cryptochironomus*.

Table 1. *Numbers of confined bluegills of two sizes killed within 1, 4, and 14 h after treatment of ponds with low and high concentrations of rotenone and antimycin.*

Pond	Treatment	Hours after treatment, total length of fish (mm), and (in parentheses) number of test fish in cages.					
		1		4		14	
		50-100 (20)	130-180 (7)	50-100 (20)	130-180 (7)	50-100 (20)	130-180 (7)
A	Control	0	0	0	1	0	1
B	Control	0	0	1	0	2	1
C	Rotenone, 0.5 mg/l	5	0	20	5	20	6
D	Rotenone, 0.5 mg/l	5	0	16	2	16	2
E	Rotenone, 2.0 mg/l	12	2	20	5	20	7
F	Antimycin, 1.5 μ g/l	0	2	5	2	6	2
G	Antimycin, 1.5 μ g/l	0	0	1	0	1	0
H	Antimycin, 10.0 μ g/l	1	0	2	0	3	0
I	Antimycin, 10.0 μ g/l	2	0	3	0	5	0

Results

Water Quality

Chemical analysis of the water from the deep well supplying the ponds used in the present experiment was given by Kennedy et al. (1970). Seasonal fluctuations in pond water chemistry (Table 4) were similar in 1971 and 1972, and did not differ from those expected in small ponds in the Midwest. Seasonal changes in alkalinity were accentuated by the development of dense stands of macrophytes. During both summers, photosynthesis resulted in the elevation of pH to values greater than 9.5 in all ponds. The rotenone and antimycin treatments had no noticeable effect on water chemistry.

Dissolved oxygen concentrations in the experimental ponds were similar to those found in many small ponds in Missouri. Summer stagnation developed in

all ponds (Table 5). Differences in surface values reflect differences in the time of sampling, which varied from about 0900 to 1300. Pond G was the only pond where anaerobiosis was detected; this condition was accompanied by the generation of hydrogen sulfide, the odor of which was evident from the pond margin.

Taxa Identified

A distinctive characteristic of the experimental waters was the dense growth of vegetation

Table 3. *Numbers of caged small (50-100 mm long) and large (130-180 mm) bluegills that died after re-treatment of ponds F-I with antimycin.*

Pond	Treatment	Hours after treatment, length of fish (mm), and (in parentheses) numbers of test fish in cages			
		6		20	
		50-100 (20)	130-180 (8)	50-100 (20)	130-180 (8)
A	Control	0	0	0	0
B	Control	0	0	0	0
C	Control	0	0	0	0
D	Control	0	0	0	0
E	Control	0	0	0	0
F	20 μ g/l	20	8	20	8
G	20 μ g/l	20	8	20	8
H	40 μ g/l	19	4	20	5
I	40 μ g/l	7	0	20	2

Table 2. *Toxicity test: mortality of bluegills, total length 50-100 mm, exposed to different concentrations of sand formulated antimycin at pH 9.3.*

Concentration (μ g/l)	Number of fish	Hours after treatment				
		4	5	6	12	24
5	10	0	0	0	a	a
10	10	0	0	0	0	0
15	10	0	0	0	2	2
20	10	2	2	5	8	8
40	10	a	7	a	10	10

^a No count made.

Table 4. Range of values for water quality determinations, June through August, 1971 and 1972, for five ponds at the Fish-Pesticide Research Laboratory, Columbia, Missouri.^a

Treatment and pond identification	Characteristic						Hardness (mg/l as Ca CO ₃)	Alkalinity (mg/l as Ca CO ₃)
	pH	Temperature (°C)		Dissolved oxygen (mg/l)				
		Surface	Bottom	Surface	Bottom			
Control								
A	7.8-9.9	24-30	21-28	5-16	0.7-15	80-141	60-150	
B	8.3-9.7	24-29	22-28	5-13	1-11	74-169	65-152	
Rotenone								
E	8.4-10.0	23-29	21-28	6-16	1-12	75-155	73-145	
Antimycin								
H	7.7-10.1	24-29	22-28	4-14	0.8-15	70-132	77-161	
I	8.9-10.3	24-28	21-26	4-15	0.6-9	65-122	77-120	

^a Hardness, alkalinity, and pH were determined only from surface samples; pH was measured at midmorning.

Table 5. Dissolved oxygen concentrations (mg/l) at surface (S) and bottom (B) in six ponds at the Fish-Pesticide Research Laboratory, Columbia, Missouri, July-September 1971.

Date (1971) and site of sample	Ponds					
	A	B	E	H	I	G
July 7						
S	15.3	12.8	15.6	10.4	11.0	12.4
B	2.5	10.0	11.0	11.2	8.2	5.4
July 20						
S	16.0	12.4	14.2	8.8	10.2	10.5
B	1.5	10.7	9.6	8.8	7.9	1.6
August 3						
S	16.0	12.9	13.7	11.4	14.4	15.0
B	1.5	9.7	2.5	11.0	8.6	1.0
August 21						
S	13.0	11.6	9.3	8.5	8.8	8.0
B	1.3	9.6	2.3	6.8	0.6	0.7
August 31						
S	14.0	13.1	14.0	11.2	10.8	5.0
B	0.7	1.6	2.0	1.4	0.8	0.0
September 14						
S	17.2	12.8	11.1	7.7	10.0	1.3
B	0.5	6.0	0.8	6.5	2.1	0.7
September 28						
S	9.9	11.6	13.2	11.6	12.2	7.8
B	6.7	3.2	2.0	3.8	3.9	4.6

throughout all of the ponds. Bushy pondweed (*Najas guadalupensis*) and chara (*Chara* sp.) were the most abundant plants. Others included water hyssop (*Bacopa rotundifolia*), smartweed (*Polygonum* sp.), arrowhead (*Sagittaria* sp.), spike rush (*Eleocharis* sp.), and sedge (*Carex* sp.).

Seventy-four animal taxa were identified (Table 6). Most abundant members of the communities were herbivorous mayflies (*Caenis simulans* and *Callibaetis fluctuans*), predaceous dragonflies (*Enallagma civile* and *Ischnura verticalis*), and predaceous midges (*Sayomyia punctipennis*, *Ablabesmyia peleenis*, and *Procladius bellus*). Other true midges were the filter feeding midges *Tanytarsus* sp. of the tribe Calopsectrini and *Pseudochironomus richardsoni* and *Chironomus attenuatus* of the tribe Chironomini. Other abundant members included ooze transporting oligochaetes and periphyton browsing snails (*Physa* sp., *Gyraulus* sp., and *Helisoma* sp.).

Effects on Abundance of Benthic Organisms

Inasmuch as the effects of treatment on benthic organisms were similar for both heavy and light applications of the toxicants, the data from heavy applications of rotenone (2 mg/l, pond E), and antimycin (4) μ g/l, ponds H and I), have been selected as representative.

Rotenon Treatment, 2 mg/l

Short-term effects.—No immediate short-term (August-September 1971) effects from application of

Table 6. *Benthic organisms collected from research ponds A-I, Fish-Pesticide Research Laboratory, Columbia, Missouri, June 1971-August 1972. The numerically dominant members of each group are indicated with an asterisk; dominant groups in number and volume are indicated by two asterisks.*

OLIGOCHAETA (aquatic earthworms)**

INSECTA

EPHEMEROPTERA (mayflies)**

Baetidae

*Caenis simulans**

*Callibaetis fluctuans**

Ephemeridae

Hexagenia bilineata

ODONATA (dragonflies and damselflies)**

Libellulidae

*Tramea carolina**

Libellula (2 species)*

*Erythemis simplicicollis**

Plathemis sp.

Aeshnidae

Anax junius

Coenagrionidae

*Enallagma civile**

*Ischnura verticalis**

Agria sp.

HEMIPTERA (true bugs)

Mesoveliidae

*Mesovelia mulsanti**

Notonectidae

Notonecta sp.

Veliidae

Microvelia sp.

Velia sp.

Hebridae

Merragata sp.

Hydrometridae

Hydrometra martini

Belostomatidae

Belostoma

TRICHOPTERA (caddis flies)

Leptoceridae

*Oecetis inconspicua**

Leptocella sp.*

Hydroptilidae

Oxyethira sp.*

Phryganeidae

Phryganea

Agrypnia

COLEOPTERA (beetles)

Hydrophilidae

Berosus sp.*

Tropisternus

Dytiscidae

*Laccophilus maculosa**

Bidessus lacustris

Hydroporus sp.

Agabus sp.

Ilybius sp.

Coptotomus

Halipilidae

Halipilus sp.*

Peltodytes

Gyrinidae

Dineutus assimilis

DIPTERA (true flies)**

Chaoboridae (phantom midges)

Chaoborus americanus

Sayomyia punctipennis

Chironomidae (true midges)

*Pseudochironomus richardsoni**

*Ablabesmyia peleenis**

Tanytarsus (2 species)*

*Chironomus attenuatus**

*Procladius bellus**

P. subletti

Labrundinia pelloso

Larsia planensis

Dicrotendipes nervosus

D. modestus

Harnischia collarator

H. monochromus

H. potamogeti

Clinotanypus pinquis

Tanypus punctipennis

T. neopunctipennis

Glyptotendipes barbipes

Endochironomus nigricans

Polypedilum simulans

P. trigonus

Lauterborniella varipennis

Psectrocladius dyari

Monopelia sp.

Cricotopus

Cryptochironomus fulvus

Corynoneura

Paratendipes

Ceratopogonidae

Two unidentified species

Stratiomyiidae

Odonotomyia sp.

Tabanidae

Chrysops sp.

Tabanus sp.

GASTROPODA (snails)**

*Gyraulus**

Physa

Heliosoma

PELECYPODA (clams)

2 mg/l rotenone in pond E were observed. Major species of mayflies, dragonflies, damselflies, aquatic earthworms, snails, phantom midges (Chaoboridae), and true midges (Chironomidae), present before treatment were also present after treatment. Although most populations of *Caenis simulans*, *Tramea carolina*, *Enallagma civile*, *Ablabesmyia peleenis* and *Pseudochironomus richardsoni* declined in abundance (Fig. 1), these downward trends had started before treatment. Results were similar in the ponds (C and D) treated with 0.5 mg/l rotenone. The declines noted were the result of emergence of insects and summer stagnation, rather than toxicity of the chemical; trends in population density were similar in control and rotenone-treated ponds. Populations of *Sayomyia punctipennis*, *Libellula* sp., *Erythemis*

simplicicollis, *Ischnura verticalis*, and *Dicrotendipes* showed no density reductions.

Long-term effects.—Long-term effects of a rotenone treatment of 2 mg/l (Pond E) were evaluated by comparing population densities in 1972 with those in 1971. The same species were present in both summers and, generally, in the same relative abundance (Fig. 1).

Although the population densities in 1972 were somewhat variable with respect to the densities measured in 1971, there was no evidence that the rotenone treatment was responsible for these variations. Rather, differences appeared to be due to natural population fluctuations.

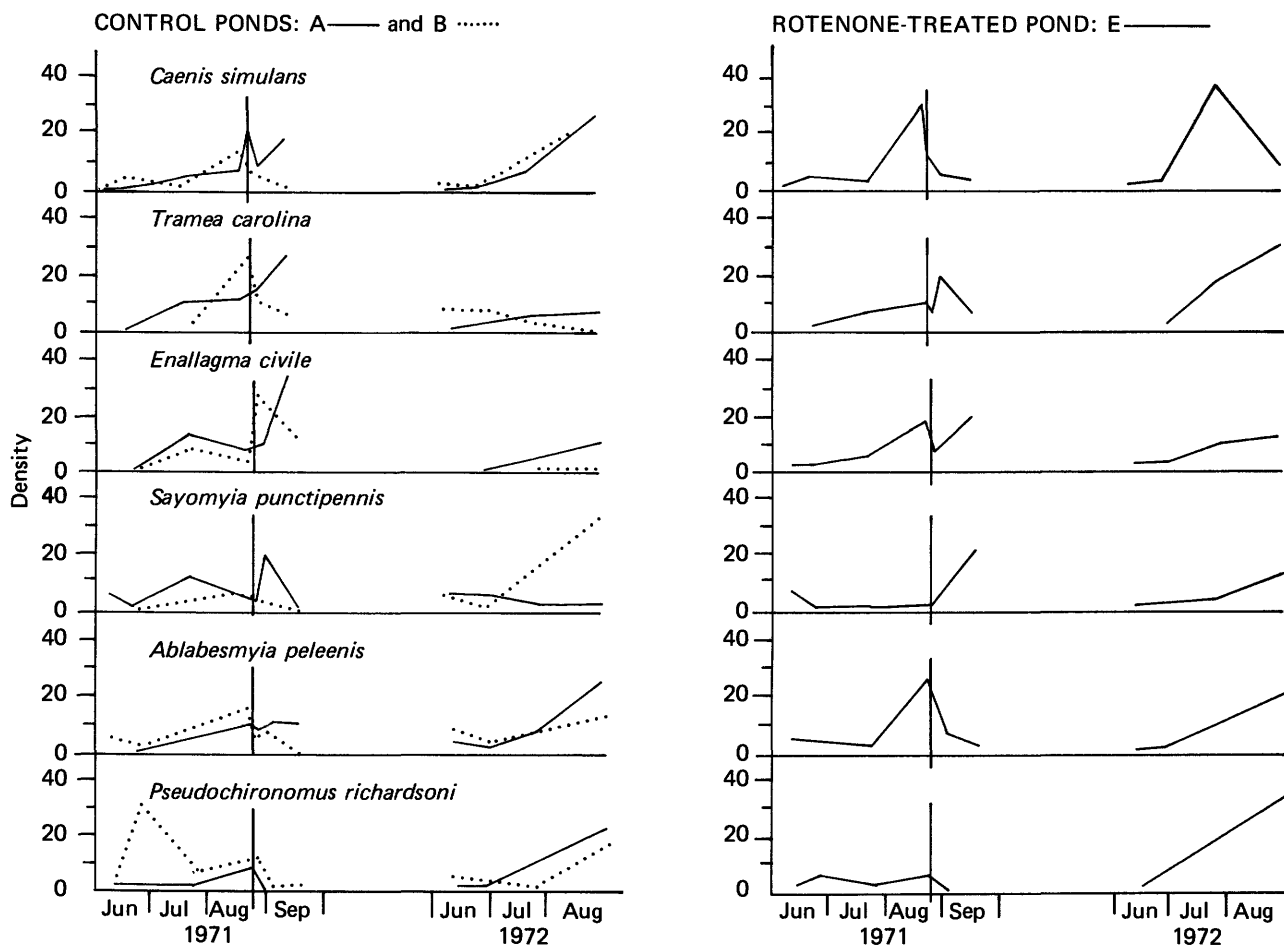


Fig. 1. Changes in population densities of major species of aquatic insects in control ponds A and B, and rotenone-treated (2 mg/l) pond E, 1971 and 1972. Vertical line in August shows treatment date. Density values express numbers collected on any one collection date as a percentage of the total numbers of that species collected throughout the period of collection.

Antimycin Treatment, 40 $\mu\text{g/l}$

Short-term effects.—No dominant organisms which were present before treatment were eliminated by exposure to heavy (40 $\mu\text{g/l}$) applications of antimycin in Ponds H and I. These dominant species included representatives of the mayflies, dragonflies, damselflies, phantom midges, true midges, snails, and aquatic earthworms.

In general, a decline in population density was observed during a brief period after treatment, in both treated and control ponds (Fig. 2). In a few organisms this decline preceded treatment. These declining populations are, therefore, not a response to toxicants but to summer stagnation (Table 5) and emergence. Populations of *Caenis simulans*, *Sayomyia punctipennis*, and *Ablabesmyia peleenis* are examples.

The relatively minor variability among some populations in control ponds and treated ponds—e.g., *Tramea carolina*, *Enallagma civile*, and *Pseudochironomus richardsoni*—does not negate this general observation.

Long-term effects.—No long-term effects on the bottom fauna were observed after applications of 40 $\mu\text{g/l}$ antimycin in Ponds H and I. This judgment is based on a comparison of the presence or absence and the densities of the major taxa in 1971 and 1972—*Caenis simulans*, *Tramea carolina*, *Enallagma civile*, *Sayomyia punctipennis*, *Ablabesmyia peleenis*, and *Pseudochironomus richardsoni* (Fig. 2)—which were selected as representative. Species present in 1971 were present in similar numbers in 1972.

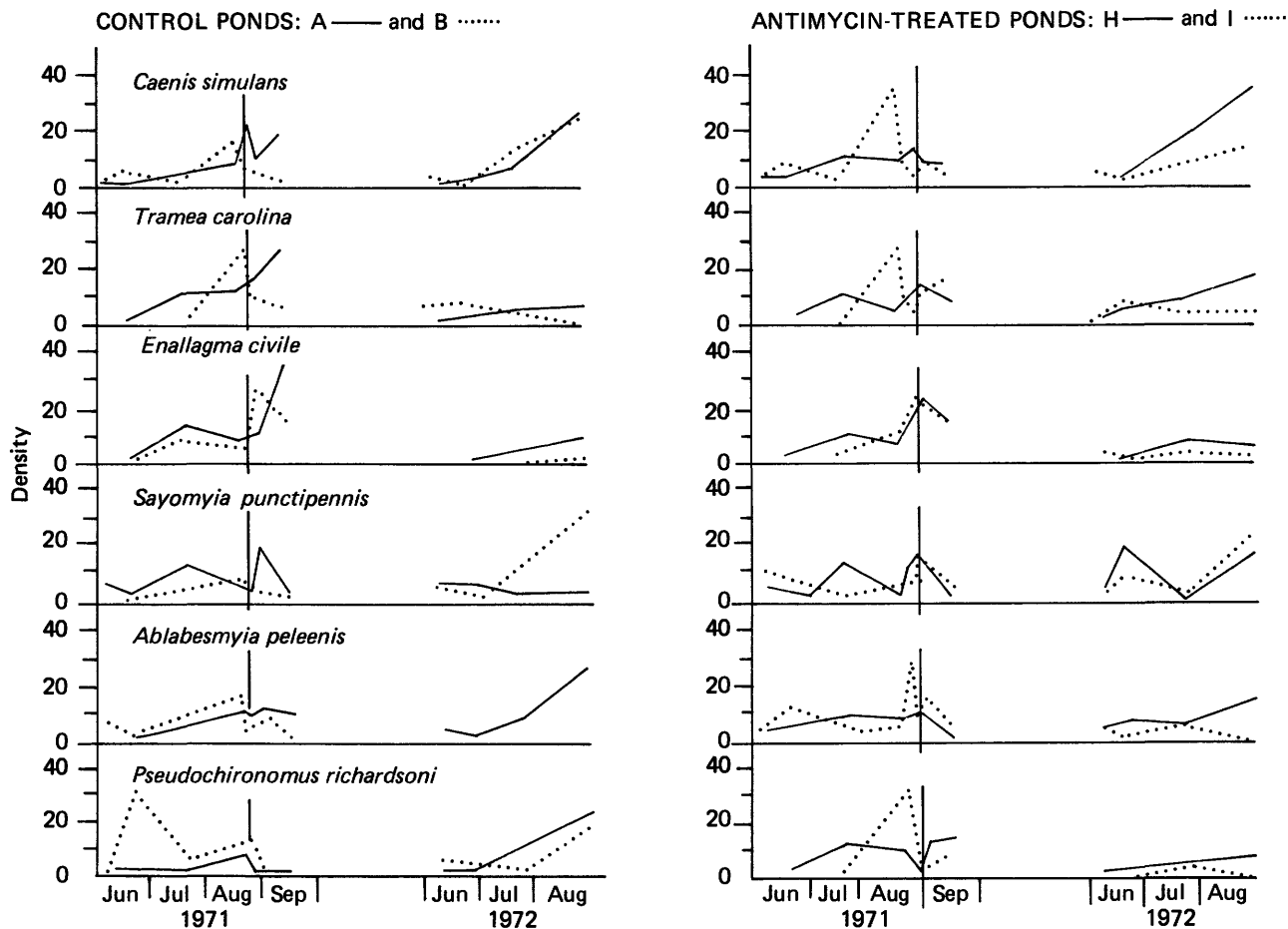


Fig. 2. Changes in population densities of major species of aquatic insects in control ponds A and B and the antimycin-treated (40 $\mu\text{g/l}$) ponds H and I, 1971 and 1972. Vertical line in August shows treatment date. Density values express numbers collected on any one collection date as a percentage of the total numbers of that species collected throughout the period of collection.

Effects on Insect Emergence

We conclude that there was no consistent evidence of toxicant interference with insect emergence, on the basis of comparisons of emergences in control ponds and in ponds treated at specified concentrations. The evaluation of emergence was made in 1972, 6 months or more after pond treatment. Emergence behavior was considered to be representative in six selected species: *Caenis simulans*, *Tramea carolina*, *Enallagma civile*, *Sayomyia punctipennis*, *Pseudochironomus richardsoni*, and *Ablabesmyia peleenis* (Table 7). The total emergence for the study period, April through June, was subdivided to show the percentage occurring in each month for each treatment, so that early or delayed emergence can be identified. Inspection of the table indicates that, although toxicants appeared to have a depressing effect in some treated ponds, comparison with control ponds in the same month shows that the decrease was instead a result of early emergence. For example,

fewer *Pseudochironomus richardsoni* or *Enallagma civile* emerged in June in treated ponds than in control ponds; however, April values indicate that emergence was early in the treated ponds and late in the control ponds.

Effects on Species Diversity

Species diversity appeared unchanged as a result of pond treatment by toxicants at specified concentrations. Diversity was judged by enumeration of the taxa and by calculation of species diversity indices in control and treated ponds.

The number of taxa present in each pond for 14 samplings in 1971 and 1972 (Table 8) did not differ consistently between (a) pre- and post-treatment samples in 1971, (b) the years 1971 and 1972, or (c) control ponds and ponds treated with antimycin and rotenone. The mean number of taxa for all ponds combined was 21.3. The mean number for all 1971 and 1972 samples for each pond was near this value.

Table 7. *Emergence of insects in ponds treated with rotenone or antimycin, and in control ponds, April-June 1972. Monthly values are expressed as percentage of the total emergence April-June for each species for each treatment.*

Species, and month of emergence	Control ponds	Treatment	
		Rotenone	Antimycin
<i>Caenis simulans</i>			
April	0	0	0
May	0	0	0
June	100	100	100
<i>Tramea carolina</i>			
April	0	0	0
May	0	14	9
June	100	86	91
<i>Enallagma civile</i>			
April	0	0	0
May	0	25	46
June	100	75	54
<i>Sayomyia punctipennis</i>			
April	0	4	1
May	67	19	1
June	33	77	98
<i>Ablabesmyia peleenis</i>			
April	8	16	10
May	50	30	23
June	42	54	67
<i>Pseudochironomus richardsoni</i>			
April	35	70	76
May	11	11	9
June	54	19	15

Table 8. The number of taxa present in control ponds and in ponds treated with high concentrations of rotenone or antimycin, Fish-Pesticide Research Laboratory, Columbia, Missouri, June 1971-August 1972.

Sampling date	Control		Treatment ^a		
			Rotenone (2 mg/l)	Antimycin (40 μ g/l)	
	Pond A	Pond B	Pond E	Pond H	Pond I
1971					
June 8	19	16	18	23	19
June 22	9	16	14	24	20
July 20	21	17	16	24	—
August 21	23	21	25	23	25
August 24	20	24	22	27	30
August 25	—	—	T	T	T
August 28	16	16	12	19	14
September 1	—	—	—	RT	RT
September 1	—	—	—	25	23
September 4	20	16	17	17	19
October 12	26	16	28	21	18
1972					
April 15	24	25	27	27	20
June 8	13	13	12	15	25
June 27	21	20	21	26	22
July 25	31	22	23	25	23
August 29	60	17	27	32	20
Mean, all samples	23.3	18.4	20.2	23.4	21.4

^a T = treatment; RT = re-treatment. Ponds H and I were re-treated with 40 μ g/l antimycin after treatments with 10 μ g/l proved to be insufficient to kill all caged fish in the pond.

Calculated species diversity indices for all sampling periods in 1971 and 1972 for each pond fell between the values 2.00 and 4.12 (Table 9).

Discussion

The results of this study justify the proposal that the toxicants antimycin and rotenone be retained as fish control agents because they are not detrimental to benthic communities when applied in proper dosages. The 14-month investigation of high and low concentrations revealed no short- or long-term effects on species abundance or on insect emergence. These conclusions are generally in agreement with published literature. Other studies have shown that species of major benthic groups have not been seriously affected by recommended treatment concentrations of 10 μ g/l antimycin (Walker et al. 1964, Gilderhus et al. 1969) or of 0.5 mg/l of rotenone (Brown and Ball 1942). However, Brown and Ball did identify an initial reduction in the population of certain unidentified species of Chaoboridae. The chaoborid *Sayomyia punctipennis* in the present

study was not affected. Penick (1963) described a study by H.S. Swingle showing that *S. punctipennis* was unaffected by rotenone.

Our analysis of species diversity indices showed that treatment with toxicants at specified concentrations did not disturb benthic communities, either immediately after treatment or in the following year. With one exception, our indices fell within the range 2.0 to 4.1. Wilhm (1970) suggested that indices below 1.0 identify unstable, disturbed benthic communities and that indices between 3.0 and 4.0 identify undisturbed communities. Applying these criteria to our indices, we conclude that none of our experimental or control ponds supported disturbed communities. The random occurrence of indices between 2.0 and 4.0 throughout control and treated ponds is further evidence that these ponds contained stable communities.

The value of species diversity indices as monitors of community stability was demonstrated by an inconsistently low index of 1.80 in pond G (treated with 20 μ g/l antimycin), which was associated with the development of anaerobic conditions (Table 5) and generation of hydrogen sulfide.

Table 9. *Diversity indices for control ponds and ponds treated with high concentrations of rotenone or antimycin, Fish-Pesticide Research Laboratory, Columbia, Missouri, June 1971-August 1972.*

Sampling date	Control		Treatment ^a		
	Pond A	Pond B	Rotenone 2 mg/l	Antimycin 40 μg/l	
			Pond E	Pond H	Pond I

1971					
June 8	2.00	2.38	2.14	2.52	3.31
June 22	2.75	2.25	3.12	2.70	2.93
July 20	2.70	3.19	2.46	3.68	b
August 21	3.60	3.60	3.34	3.62	3.66
August 24	2.81	3.56	3.26	3.80	3.48
August 25	—	—	T	T	T
August 28	3.21	2.40	3.12	3.06	2.66
September 1	—	—	—	RT	RT
September 4	—	—	—	2.66	3.76
September 14	3.05	2.68	3.08	2.93	2.87
October 12	2.45	2.62	3.51	2.74	3.12
1972					
April 15	2.83	3.42	3.74	2.68	3.57
June 8	2.06	2.93	2.24	3.12	2.74
June 27	2.21	2.54	2.54	3.37	2.97
July 25	4.12	2.77	3.24	3.61	2.38
August 29	3.26	3.33	3.56	3.53	3.02

^a T = treatment; RT = re-treatment. Ponds H and I were retreated with 40 μ g/l antimycin after treatments with 10 μ g/l proved to be insufficient to kill all caged fish in the pond.

^b Sample not analyzed.

Two environmental factors that influenced this study were the large beds of vegetation and the absence of fish. The lack of fish may modify species abundance and composition because of the accompanying marked reduction in predation on benthic organisms.

The invasion of all open water of all ponds by vegetation by late summer was accompanied by an increase in number of niches, which was in turn reflected in an increase in the species diversity index. Periods of spring emergence, summer buildup, and fall emergence of insects were closely tied to the seasonal development and decline of vegetation. Photosynthetic activity of vegetation resulted in high pH (above 9.5 in early afternoon), which caused inactivation and subsequent detoxification of antimycin, and made it necessary to increase the concentration of antimycin applied. Decomposition of plant material resulted in low dissolved oxygen concentrations in late summer (Table 5), which, in combination with insect emergence, resulted in a seasonal decline in insect abundance and a decrease in the diversity index.

An advantage of the toxicants antimycin and rotenone is that they are naturally occurring com-

pounds whose persistence is prevented through biodegradation. Thus insects, which have the capacity for rapid recolonization, are not excluded from aquatic ecosystems for long periods even after overdoses of these toxicants.

Conclusions

1. No short- or long-term effects on abundance of dominant benthic species could be attributed to pond treatments with 0.5 and 2.0 mg/l concentrations of rotenone or 20 and 40 μ g/l concentrations of antimycin.
2. Species diversity within the benthic community, as evaluated by number of taxa and diversity indices, was not changed by rotenone or antimycin treatment.
3. Insect emergence was not affected by rotenone or antimycin treatments.

Acknowledgments

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Appendices

Appendix I, Community Dynamics in the Experimental Ponds, provides a description of the dynamics of benthic populations in ponds which are heavily vegetated and which lack fish. The absence of fish may modify species abundance and composition because of the accompanying marked reduction in predation on benthic organisms. The dense vegetation provides unusual cover, more niches, and presumably more food than may be available to benthos in nonvegetated habitats. Periods of spring emergence, summer buildup, and fall emergence of insects were closely associated with the seasonal development and decline of vegetation.

Appendix II, Changes in Density of Benthic

Organisms, presents the field data from all collections, plotted as number of organisms per square meter, June-October 1971 and April-August 1972, for control and toxicant-treated ponds. Values for representative species from these data (Figs. A3-A13) provided the basis for discussion and conclusions in the text.

Appendix III, Density of Midges Captured in Emergence Cages, shows the rate of capture (no./m²/wk) of two genera of phantom midges and 24 taxa of true midges in emergence cages, April-June 1972, in two control ponds, in one pond treated with 2.0 mg/l of rotenone, and in two ponds treated with 40 μ g/l of antimycin (Figs. A14-A18).

Appendix I

Community Dynamics in the Experimental Ponds

Our observations of seasonal changes in benthic populations describe community dynamics in ponds with extensive beds of vegetation and without fishes. Seasonal changes are presented in the following sequence: summer development, fall emergence, winter development, and spring emergence. These periods were not synchronous in all ponds but were closely approximated.

Summer development was characterized by rapid larval development, as a result of high temperature. This developmental period was disrupted in the first year of the study (1971) by the draining and refilling of the ponds in late April. Draining delayed the growth of macrophytes, increased bottom organic matter and subsequent growth of benthic algae, and later resulted in extremely dense populations of *Chironomus attenuatus* and *Glyptotendipes barbipes*. These species constituted almost the entire community of benthos. Their subsequent reduction followed the decline in benthic algae as developing beds of vegetation limited light penetration.

By mid-July most major genera typical of these pond communities (Table 6) were present, and the vegetation, which developed rapidly because the carbonate reserve was high, provided an abundant food source and a variety of niches for recolonization. This recolonization was hastened by the proximity of the study ponds to nearby ponds that had not been drained.

Distinctive trends occurred in the seasonal patterns of appearance and abundance for major members of the pond communities (Figs. A1 and A2). The mayfly *Caenis simulans*, the dragonfly

Erythemis simplicicollis, and the damselfly *Ischnura verticalis* showed late summer population increases. Of the true midges, *Ablabesmyia peleenis*, *Pseudochironomus richardsoni*, *Tanytarsus*, and *Procladius subletti* showed two emergence periods and a population buildup in midsummer.

The fall emergence period was characterized by a decline in larval populations. The decline was also associated with a partial decline and decomposition of vegetation, which caused high oxygen demand. The decline in midge larvae (*Ablabesmyia peleenis*, *Pseudochironomus richardsoni*, *Procladius subletti*, and *Tanytarsus*) was due to emergence (Appendix III, Figs. A14-A18). Reduction of other species was linked with stagnation.

The winter development period was characterized by slow larval development resulting from low temperature. Initially, the numbers of mayflies, dragonflies, and damselflies appeared to be large. Later, the numbers declined as larval size increased. A reverse trend characterized the true midges; densities were low in late October and high in early April (Appendix II, Figs. A3-A13).

The spring emergence period was characterized by intensified emergence of genera that pupate throughout the year, and also by the emergence of the genera that pupate once a year. The highly distinctive seasonal emergence pattern of the true midges (Chironomidae) is described for the months of April, May, and June in Table A1. The total emergence resulted in the smallest number of individuals and in the lowest values for species diversity indices found throughout the year.

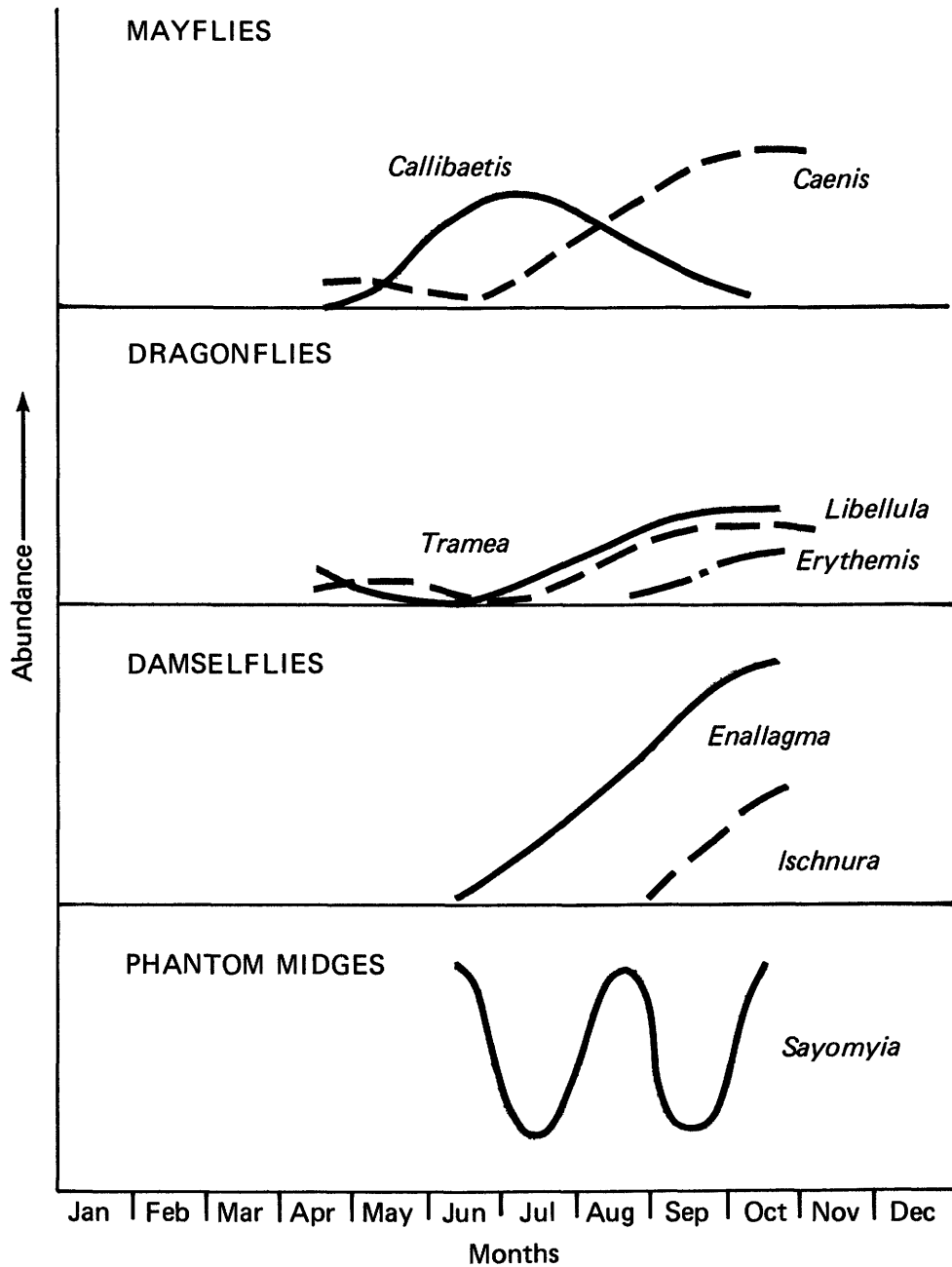


Fig. A1. Schematic presentation of trends in seasonal abundance of the most common dragonflies, damselflies, and phantom midges, collected from nine experimental ponds (A-I) near Columbia, Missouri, in 1971.

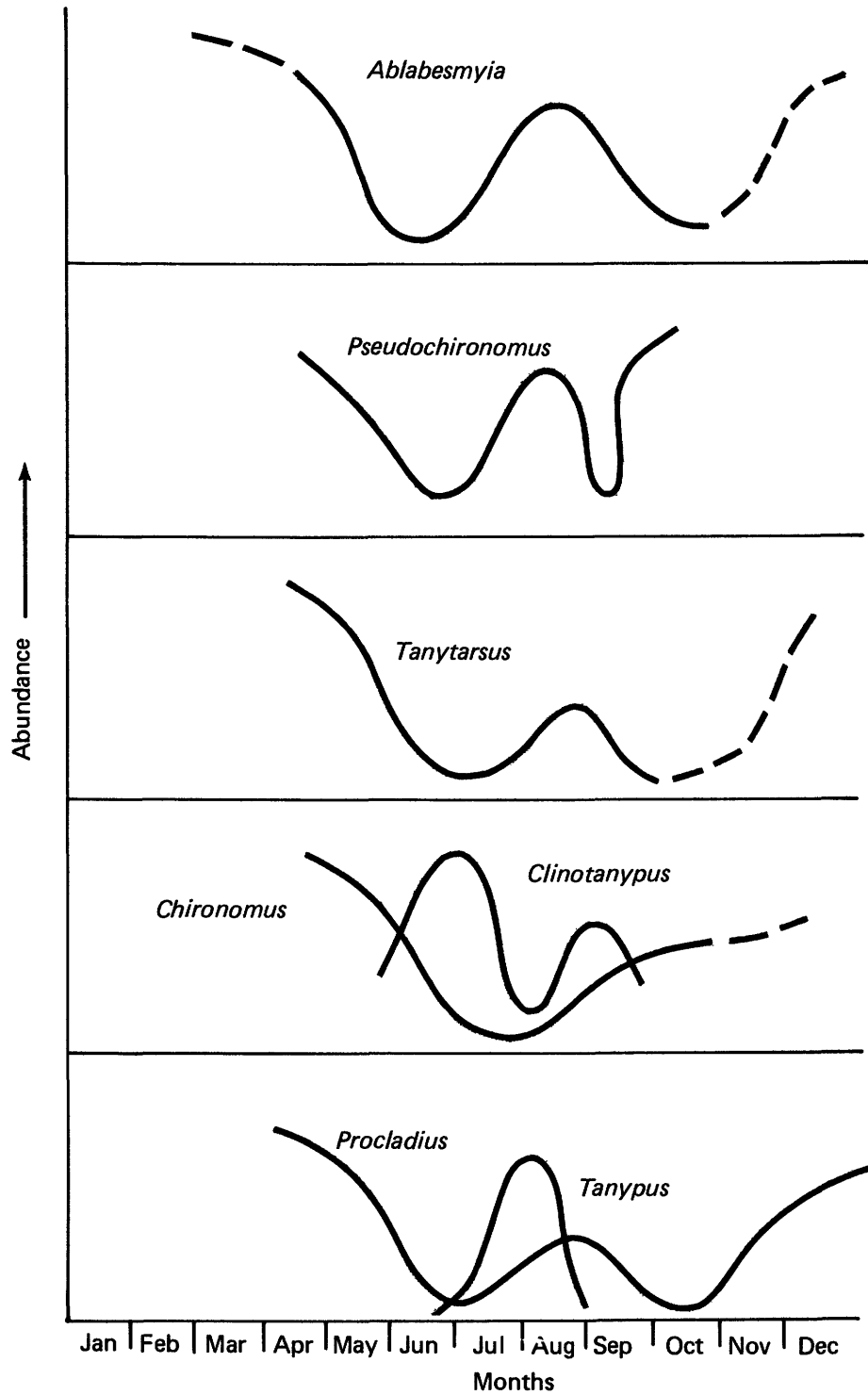


Fig. A2. Schematic presentation of trends in seasonal abundance of seven of the most common midges collected from nine experimental ponds (A-I) near Columbia, Missouri, in 1971.

Table A1. Seasonal emergence patterns for midges (*Chironomidae*) in experimental fish ponds A to I, Fish-Pesticide Research Laboratory, Columbia, Missouri, April-June 1972. Presence of identifying letter indicates emergence from that pond.

Species	April	May	June
<i>Pseudochironomus richardsoni</i>	ABEHI	ABEHI	ABEHI
<i>Tanytarsus</i> sp.	ABEHI	ABEHI	A - EHI
<i>Dicrotendipes nervosus</i>	--EHI	A - EHI	ABEHI
<i>Procladius bellus</i>	ABEHI	-BEHI	ABEHI
<i>Ablabesmyia peleenis</i>	ABEHI	ABEHI	ABEHI
<i>Endochironomus nigricans</i>	--E--	A----	A -- H-
<i>Psectrocladius dyari</i>	-B---	-----	-BE--
<i>Monopelia</i> sp.	-----	A -- HI	A - EH-
<i>Lauterborniella varipennis</i>	-----	ABEHI	ABEHI
<i>Larsia planesis</i>	-----	ABEHI	ABEHI
<i>Clinotanypus pinguis</i>	A----	ABEH-	ABEHI
<i>Glyptotendipes barbipes</i>	-----	AB---	A----
<i>Labrundinia pelloso</i>	-----	--EHI	-BEHI
<i>Polypedilum simulans</i>	--E--	--EHI	ABEHI
<i>Chironomus attenuatus</i>			---HI

Appendix II

Changes in Density of Benthic Organisms in Heavily Vegetated Ponds Lacking Fish Populations, June-October 1971 and April-August 1972

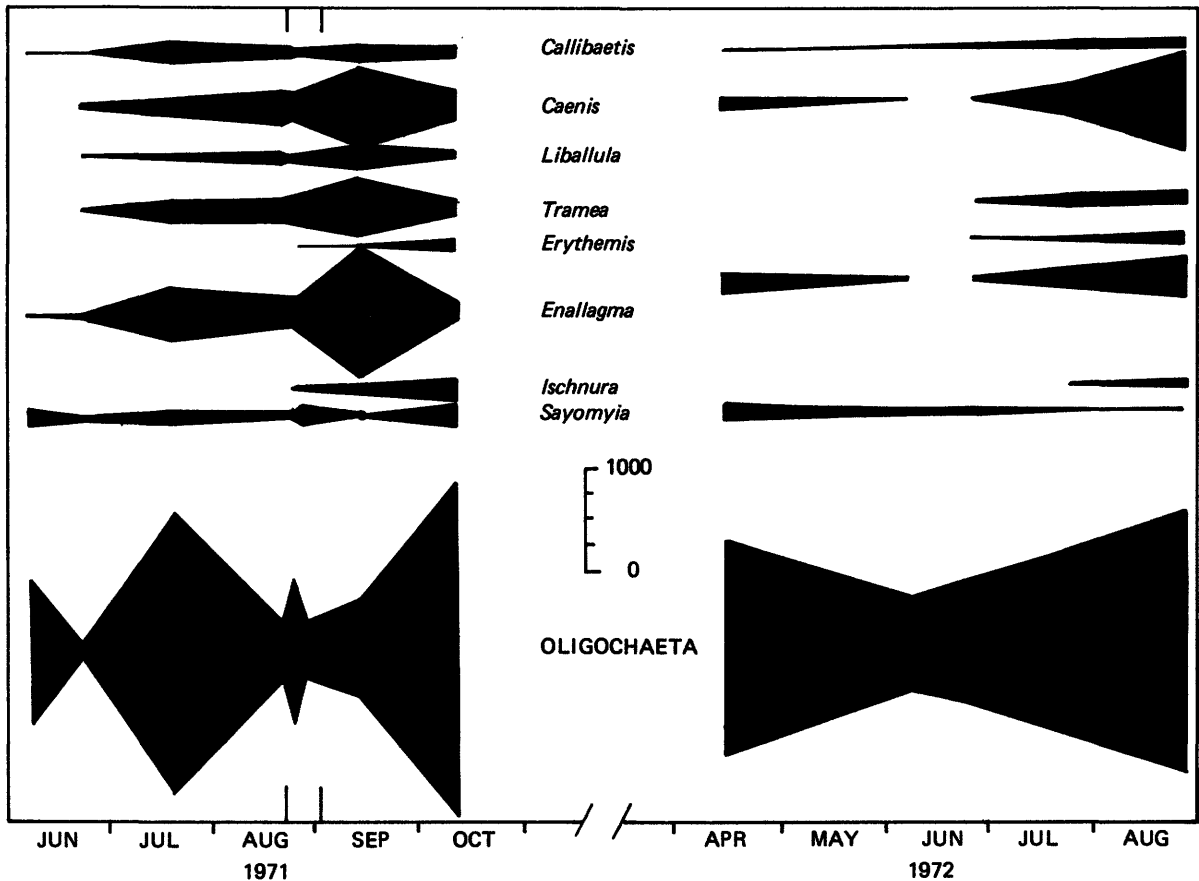


Fig. A3. Changes in density (no./m²) of mayflies (*Callibaetis*, *Caenis*), dragonflies (*Libellula*, *Tramea*, *Erythemis*), damselflies (*Enallagma*, *Ischnura*), phantom midge (*Sayomyia*), and aquatic earthworms (*Oligochaeta*) in control Pond A at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.

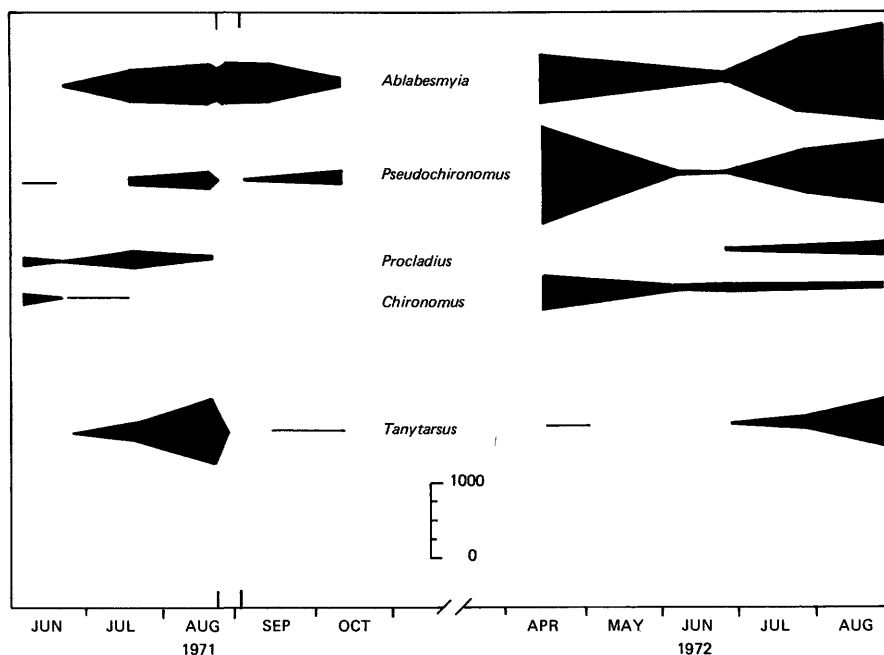


Fig. A4. Changes in density (no./m²) of midges (*Ablabesmyia*, *Pseudochironomus*, *Procladius*, *Chironomus*, *Tanytarsus*) in control Pond A at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.

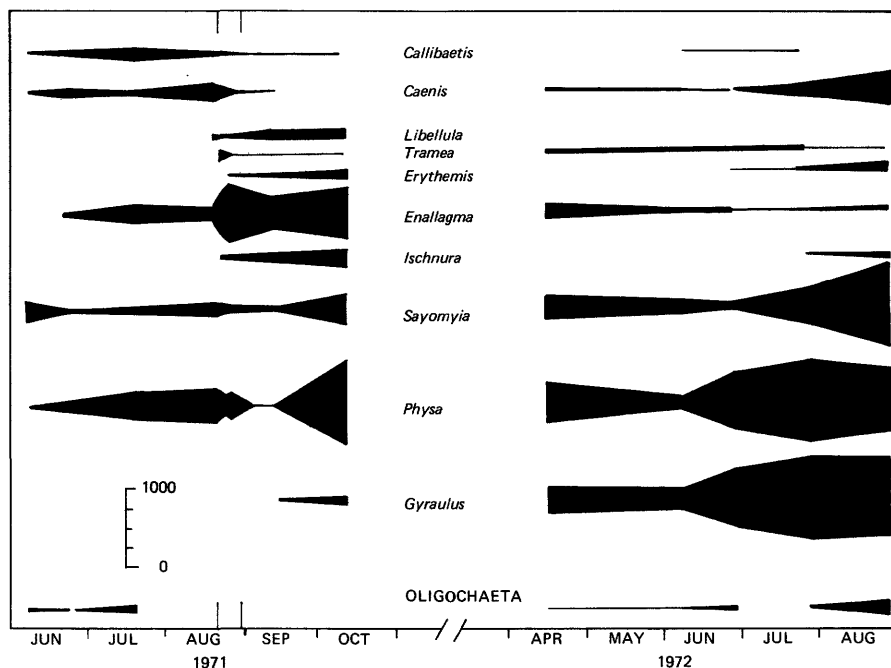


Fig. A5. Changes in density (no./m²) of mayflies (*Callibaetis*, *Caenis*), dragonflies (*Libellula*, *Tramea*, *Erythemis*), damselflies (*Enallagma*, *Ischnura*), phantom midge (*Sayomyia*), snails (*Physa*, *Gyraulus*) and aquatic earthworms (Oligochaeta) in control Pond B at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.

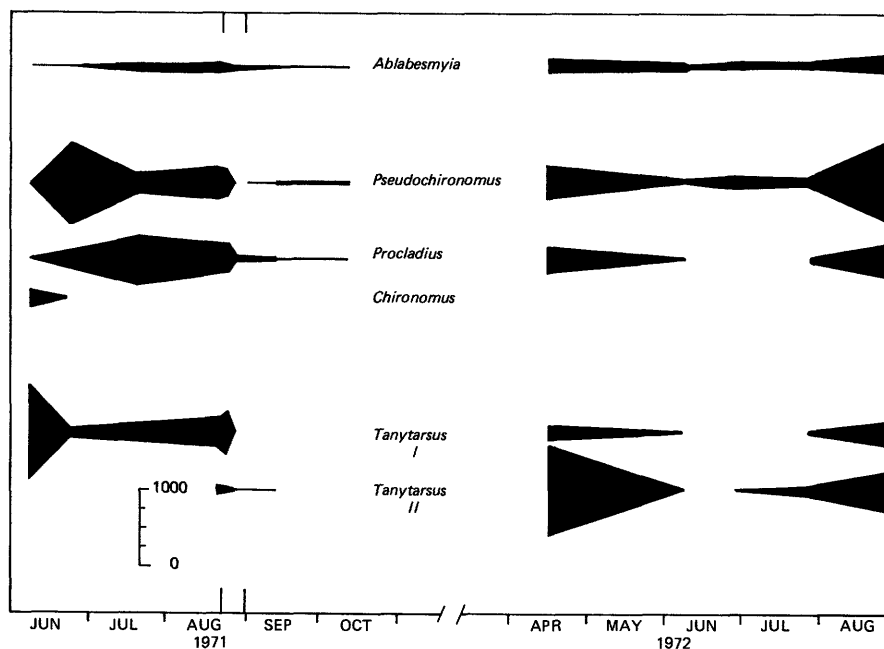


Fig. A6. Changes in density (no./m²) of midges *Ablabesmyia*, *Pseudochironomus*, *Procladius*, *Chironomus*, and two unidentified species of *Tanytarsus* in control Pond B at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.

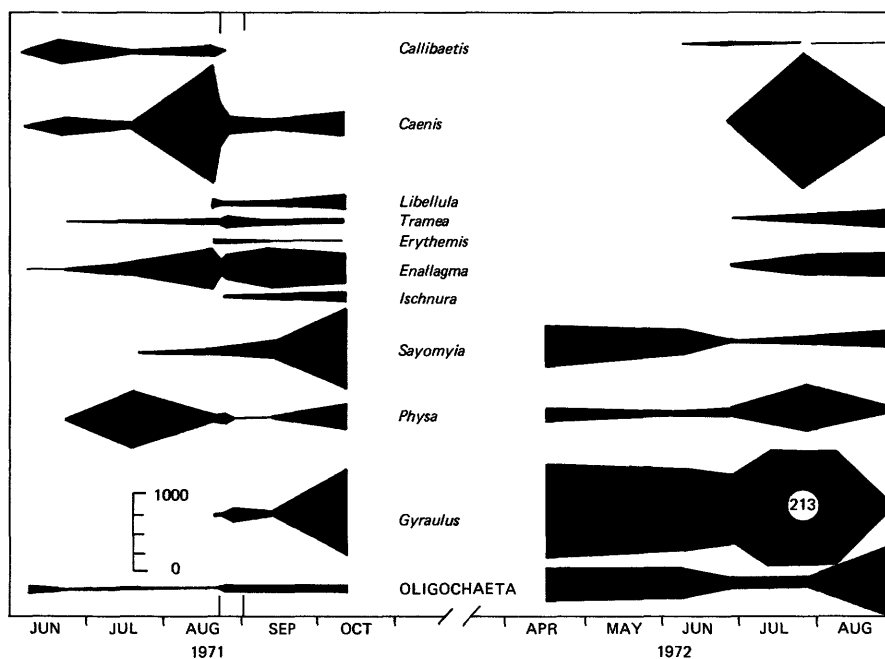


Fig. A7. Changes in density (no./m²) of mayflies (*Callibaetis*, *Caenis*), dragonflies (*Libellula*, *Tramea*, *Erythemis*), damselflies (*Enallagma*, *Ischnura*), phantom midge (*Sayornia*), snails (*Physa*, *Gyraulus*) and aquatic earthworms (*Oligochaeta*) in Pond E, treated with 2 mg/l of rotenone, at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.

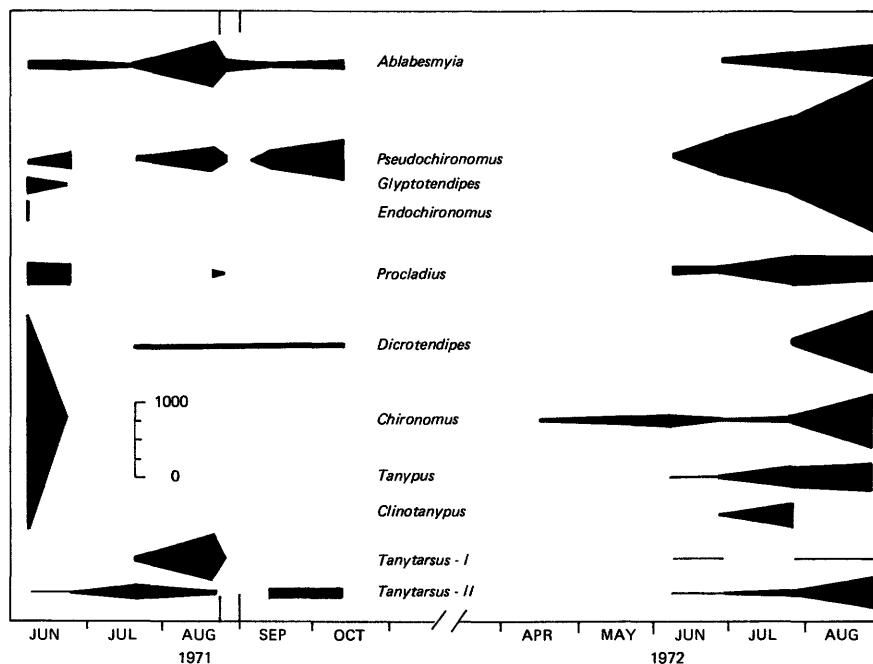


Fig. A8. Changes in density (no./m²) of midges *Ablabesmyia*, *Pseudochironomus*, *Glyptotendipes*, *Endochironomus*, *Procladius*, *Dicortendipes*, *Chironomus*, *Tanytus*, *Clinotanytus*, and two unidentified species of *Tanytarsus* in Pond E, treated with 2 mg/l rotenone, at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.

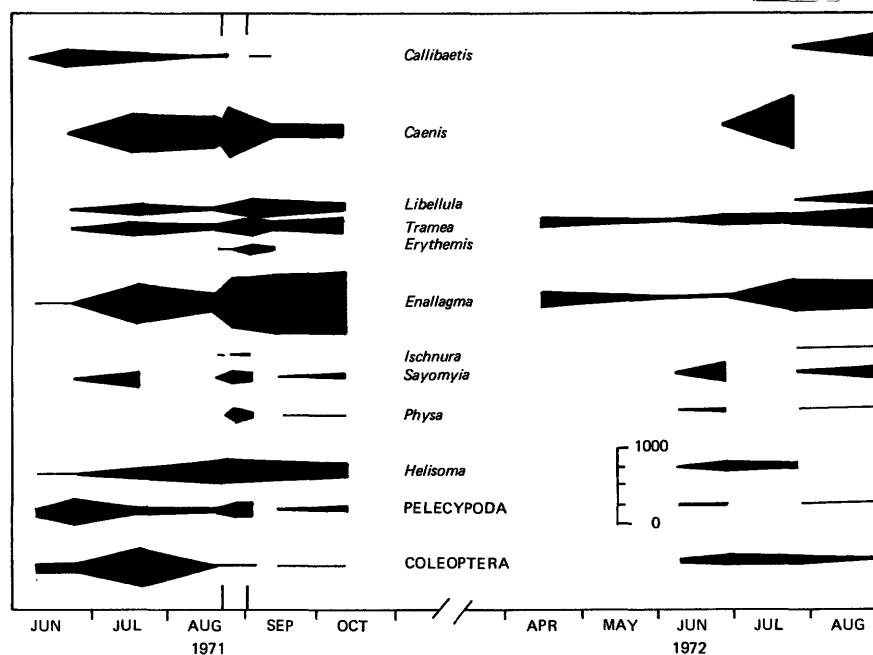


Fig. A9. Changes in density (no./m²) of mayflies (*Callibaetis*, *Caenis*), dragonflies (*Libellula*, *Tramea*, *Erythemis*), damselflies (*Enallagma*, *Ischnura*), phantom midge (*Sayomyia*), snails (*Physa*, *Helisoma*), clam (Pelecypoda) and beetles (Coleoptera) in pond H treated with 40 µg/l of antimycin at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.

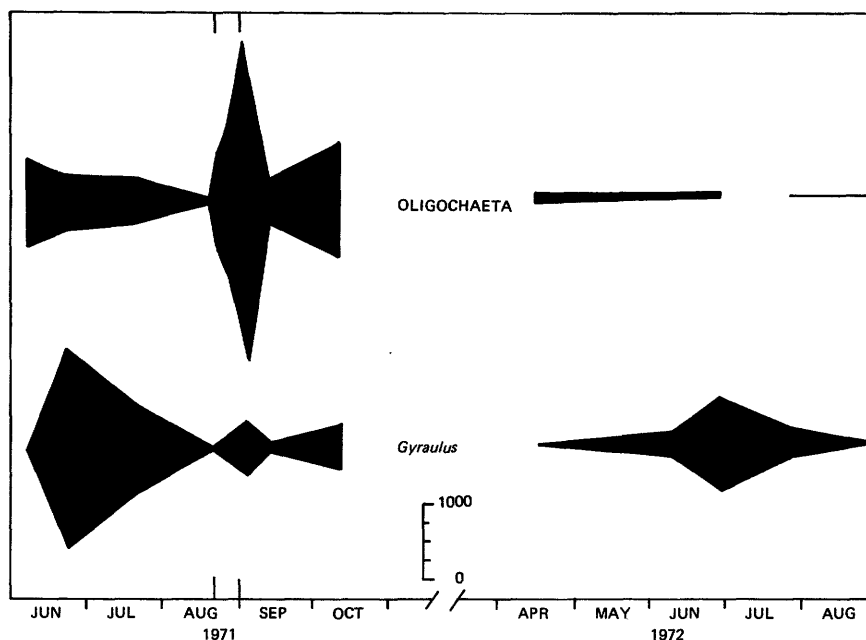


Fig. A10. Changes in density (no./m²) of aquatic earthworms (*Oligochaeta*) and snails (*Gyraulus*) in Pond H, treated with 40 μ g/l of antimycin, at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.

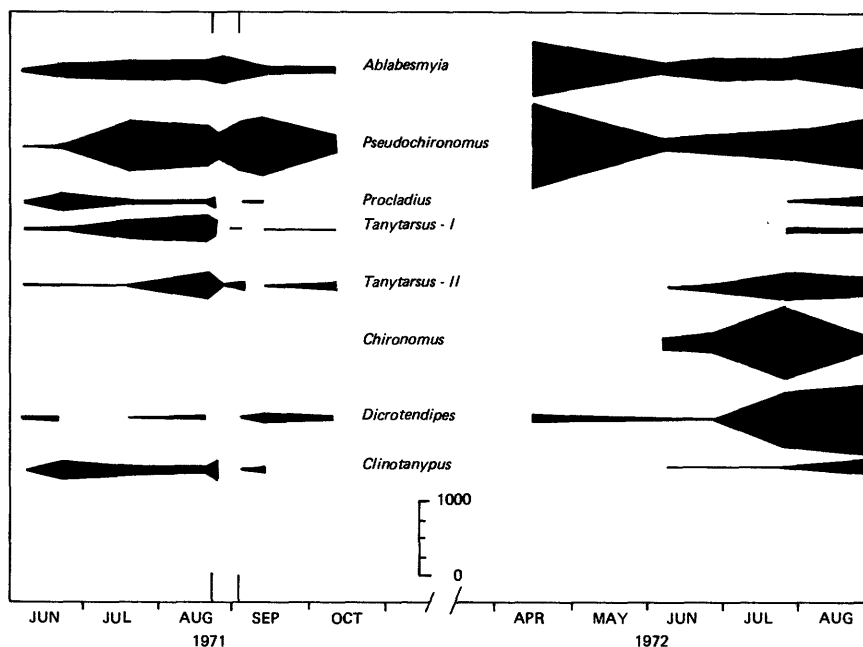


Fig. A11. Changes in density (no./m²) of midges *Ablabesmyia*, *Pseudochironomus*, *Procladius*, *Chironomus*, *Dicrotendipes*, *Clinotanypus*, and two unidentified species of *Tanytarsus* in Pond H, treated with 40 μ g/l of antimycin, at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars on the baseline indicate time of application of toxicants in the treated ponds.

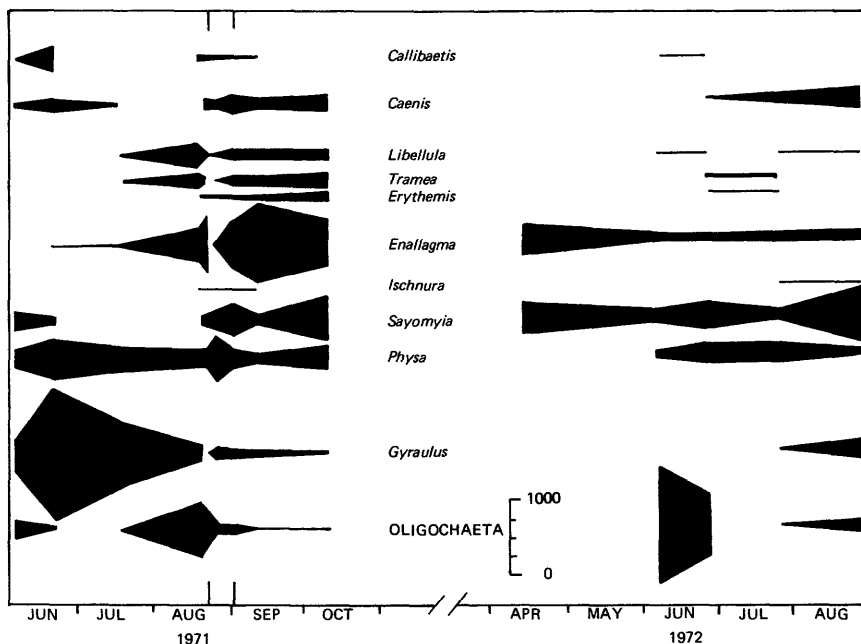


Fig. A12. Changes in density (no./m²) of mayflies (*Callibaetis*, *Caenis*), dragonflies (*Libellula*, *Tramea*, *Erythemis*), damselflies (*Enallagma*, *Ischnura*), phantom midge (*Sayomyia*), snails (*Physa*, *Gyraulus*), and aquatic earthworms (*Oligochaeta*) in Pond I, treated with 40 µg/l of antimycin, at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.

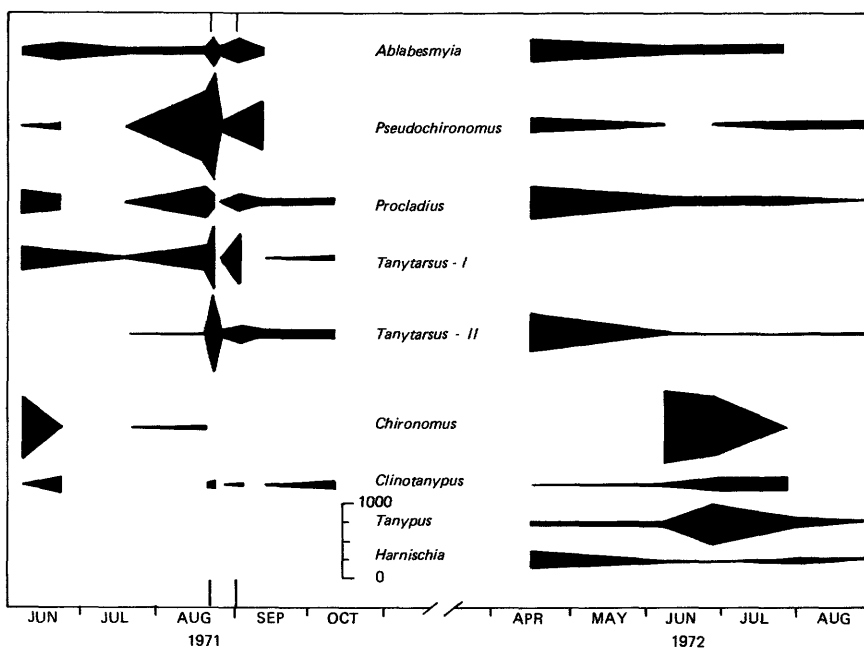


Fig. A13. Changes in density (no./m²) of midges *Ablabesmyia*, *Pseudochironomus*, *Procladius*, *Chironomus*, *Clinotanytus*, *Tanypus*, *Harnischia*, and two unidentified species of *Tanytarsus* in Pond I, treated with 40 µg/l of antimycin, at the Fish-Pesticide Research Laboratory, Columbia, Missouri, June-October 1971 and April-August 1972. The short vertical bars along the baseline indicate time of application of toxicants in the treated ponds.

Appendix III

Density of Phantom Midges and True Midges Captured in Emergence Cages in Two Control and Three Treated Ponds, April-June 1972

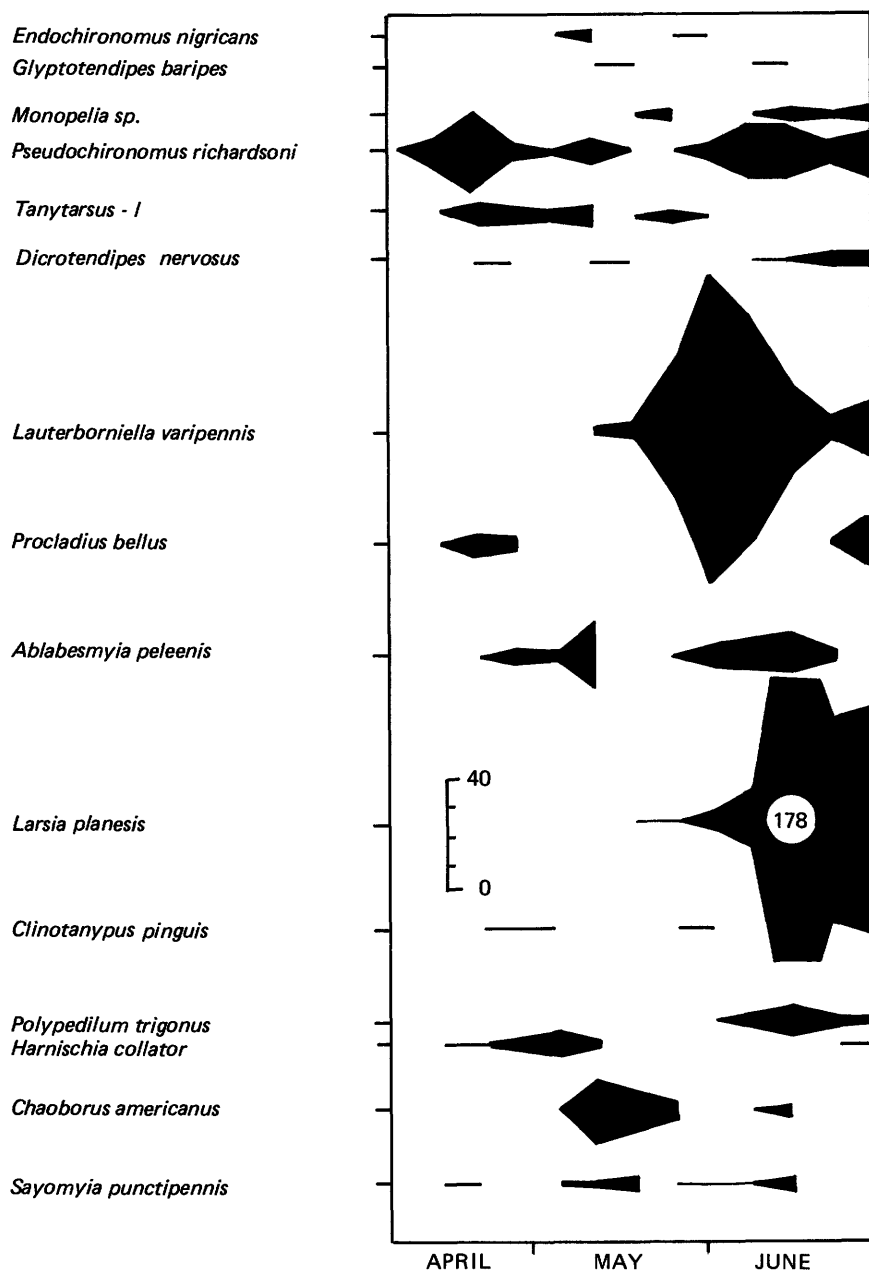


Fig. A14. Density (no./m² per week) of phantom midges (*Chaoborus* and *Sayomyia*) and true midges (other taxa shown) captured in emergence cages in control Pond A at the Fish-Pesticide Research Laboratory, Columbia, Missouri, April-June 1972. (The figure includes one unidentified species of *Tanytarsus*.)

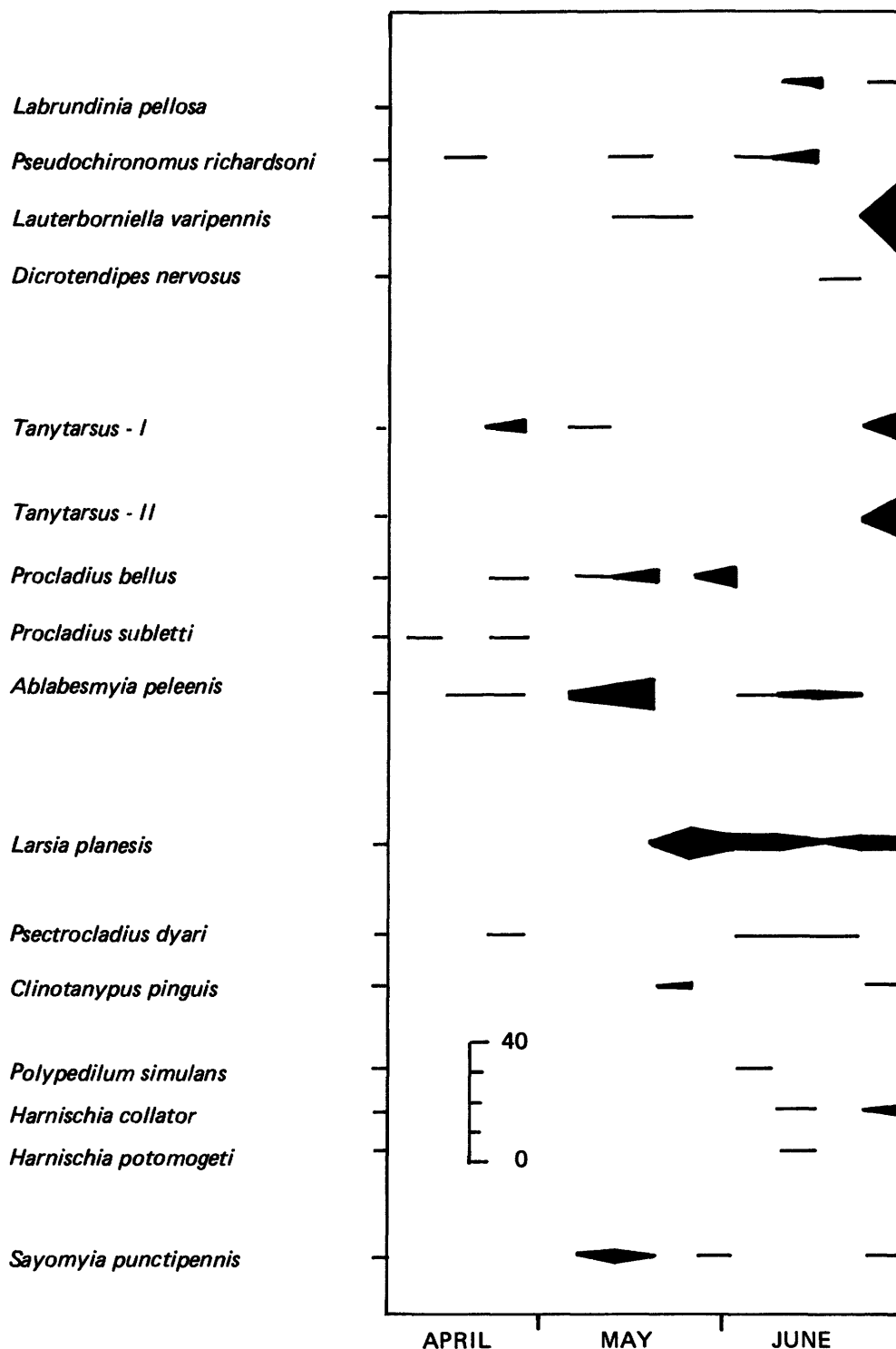


Fig. A15. Density (no./m² per week) of phantom midges (*Sayomyia*) and true midges (other taxa shown) captured in emergence cages in control Pond B at the Fish-Pesticide Research Laboratory, Columbia, Missouri, April-June 1972. (The figure includes two unidentified species of *Tanytarsus*.)

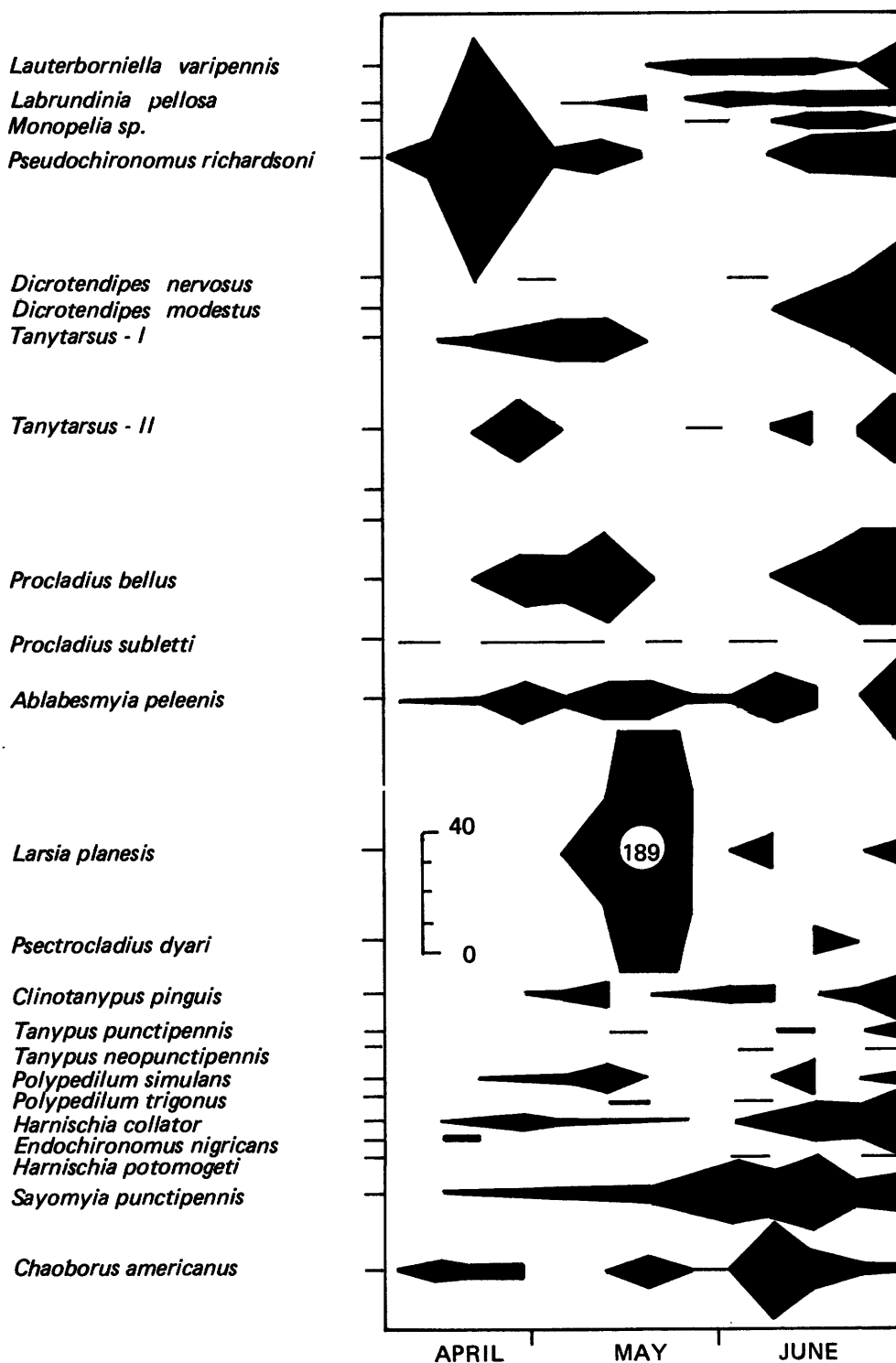


Fig. A16. Density (no./m² per week) of phantom midges (*Sayomyia*, *Chaoborus*) and true midges (other taxa shown) captured in emergence cages in Pond E, treated with 2.0 mg/l of rotenone, at the Fish-Pesticide Research Laboratory, Columbia, Missouri, April-June 1972. (The figure includes two unidentified species of *Tanytarsus*.)

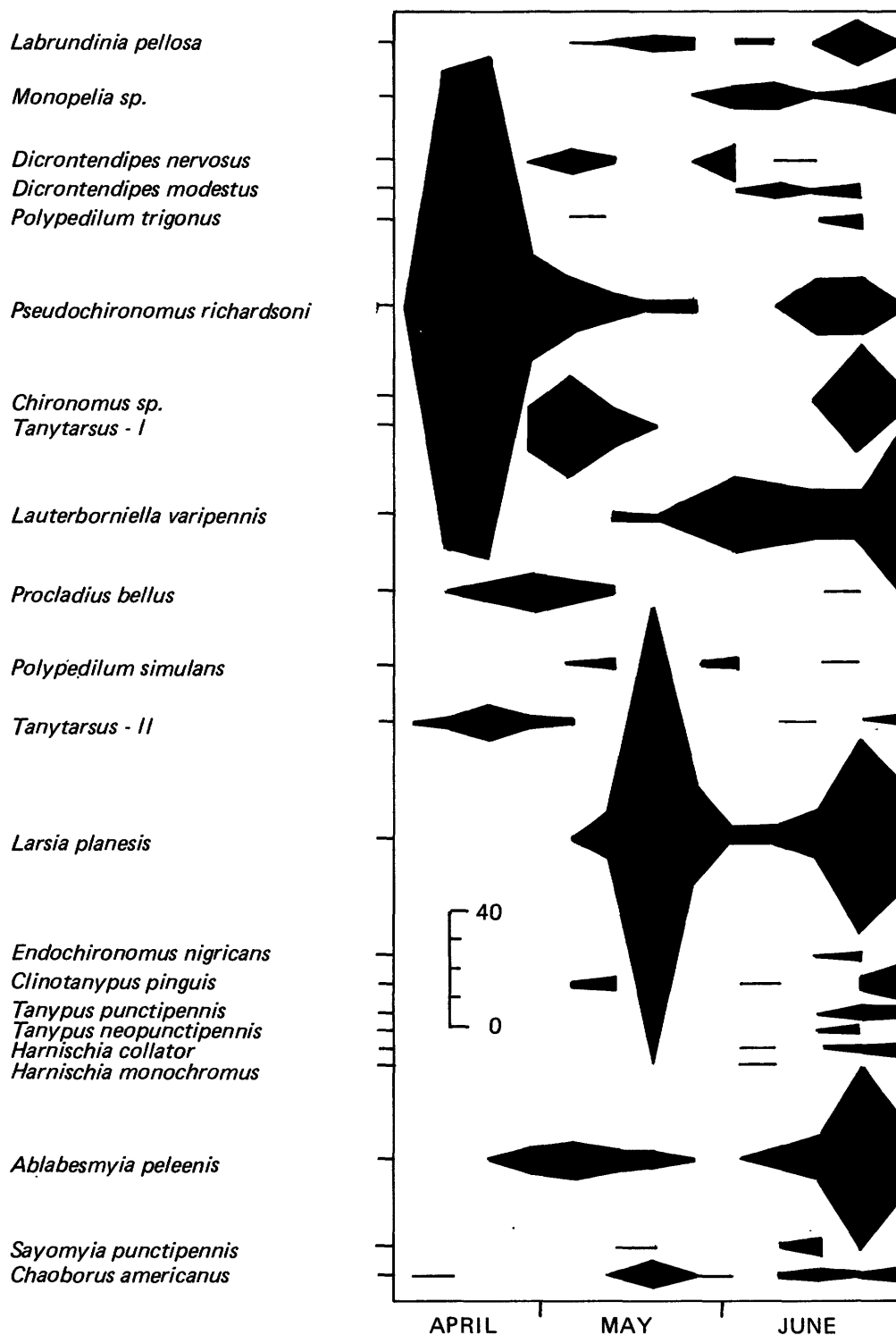


Fig. A17. Density (no./m² per week) of phantom midges (*Sayomyia*, *Chaoborus*) and true midges (other taxa shown) captured in emergence cages in Pond H, treated with 40 μ g/l of antimycin, at the Fish-Pesticide Research Laboratory, Columbia, Missouri, April-June 1972. (The figure includes two unidentified species of *Tanytarsus*.)

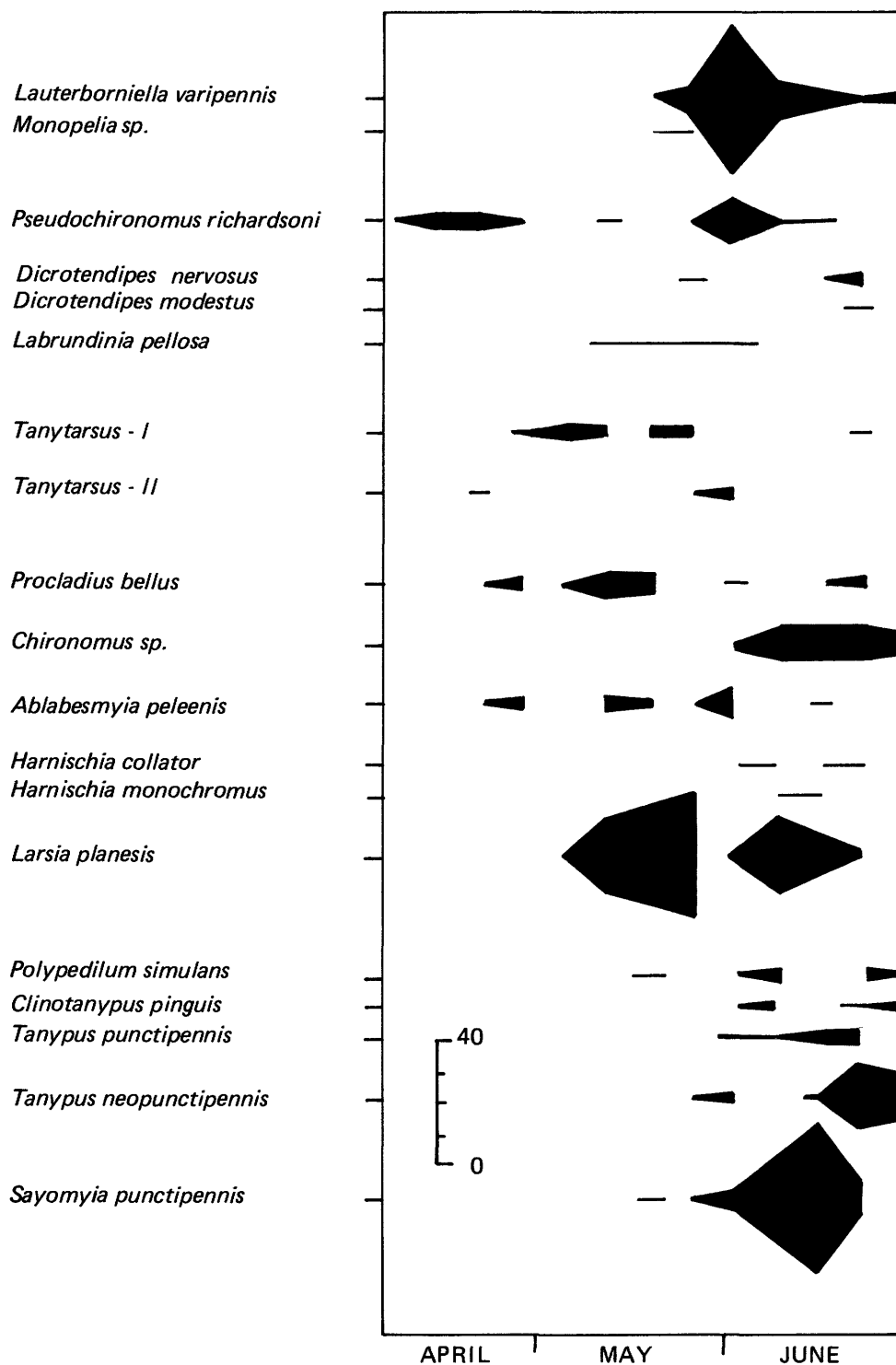


Fig. A18. Density (no./m² per week) of phantom midge (*Sayomyia*) and true midges (other taxa shown) captured in emergence cages in Pond I, treated with 40 µg/l of antimycin, at the Fish-Pesticide Research Laboratory, Columbia, Missouri, April-June 1972. (The figure includes two unidentified species of *Tanytarsus*.)

Aquatic Macroinvertebrates in a Small Wisconsin Trout Stream Before, During, and Two Years After Treatment with the Fish Toxicant Antimycin¹

by

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Abstract

Benthos and benthic drift were sampled periodically in Seas Branch Creek (Vernon County, Wisconsin) for 5 months before and for 2 years after the stream was treated with antimycin, and over the same period in nearby untreated Maple Dale Creek. During treatment on 4 October 1972, antimycin concentrations varied from 17 to 44 $\mu\text{g/l}$ at the two sampling stations in Seas Branch Creek. Populations of macroinvertebrates were drastically reduced 2 days after treatment, but all common taxa identified before treatment were present in the stream 1 year later. Estimated biomass reductions of living organisms 2 days after treatment were as high as 50% for one caddis fly, *Hydropsyche* sp., and 75% for another, *Brachycentrus americanus*; 70% for a crane fly, *Antocha* sp.; and nearly 100% for a mayfly, *Baetis cingulatus*, and a scud, *Gammarus pseudolimnaeus*. Summer biomass of *Antocha* and *Brachycentrus* did not regain pretreatment levels during the second year. The mortality of the riffle beetle, *Optioservus fastiditus*, was approximately 20% 9 days after treatment. A crayfish, *Orconectes propinquus*, was not affected by the treatment. The biomass of *Gammarus*, *Prosimulium* (a black fly), *Baetis*, and *Hydropsyche* was high during both summers after treatment. After 1 year, and continuing into the second year, total benthic biomass approached or exceeded that before treatment.

The piscicide antimycin is used for several purposes in fishery management, including eradication of nongame fish species that are suspected of competing with game fish. Antimycin and rotenone are the only two chemicals registered for such use by the Environmental Protection Agency.

In 1972 the Wisconsin Department of Natural Resources, in rehabilitating Seas Branch Creek, used antimycin to eradicate populations of catostomids and cyprinids. After removal of the nongame species, the stream was restocked with brown trout (*Salmo trutta*). This project afforded us the opportunity to observe the reactions of fish food organisms to antimycin.

The purpose of our study was to observe and document changes in nontarget aquatic macroinvertebrate populations in this small trout stream after the application of antimycin. Short- and

long-term effects of treatment were shown by quantitative and qualitative variations in benthic biomass and changes in the composition and abundance of drift organisms.

The effects of antimycin on the invertebrate fauna have been previously investigated in lakes or ponds, but not (to our knowledge) in a natural trout stream. Callahan and Huish (1969) and Rabe and Wissman (1969) reported that 5.0 $\mu\text{g/l}$ applications of antimycin severely reduced populations of zooplankton in lakes and ponds, whereas Walker et al. (1964), Gilderhus et al. (1969), and Houf and Hughey (1973) found that fish-killing concentrations of antimycin had no significant effect on lake plankton and benthos. Snow (1974) observed no gross long-term detrimental effects on zooplankton and benthos 6 years after antimycin treatment in Rush Lake, Wisconsin.

Study Area

Seas Branch Creek is in central Vernon County, in the hilly, unglaciated area of southwestern Wisconsin (Fig. 1). It is an 8-km-long tributary of the West

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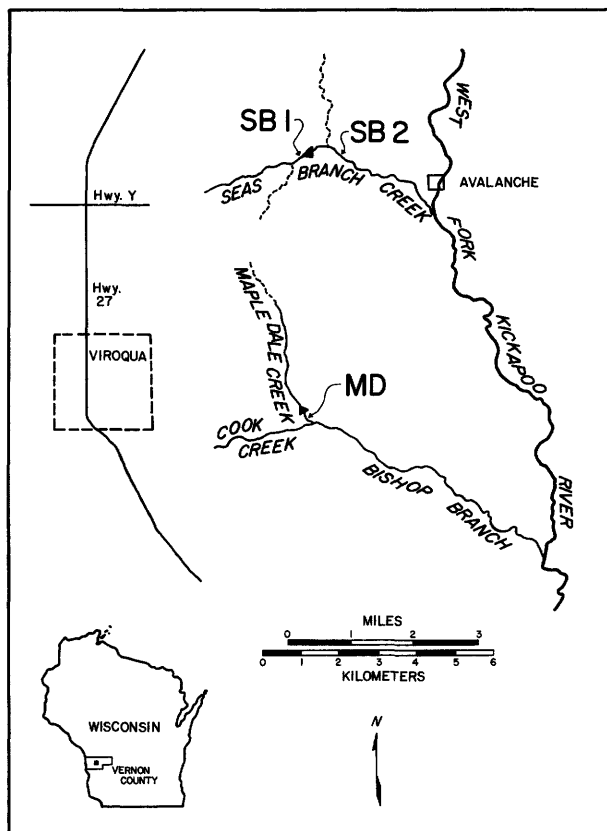


Fig. 1. Locations of study stations SB1 and SB2 on Seas Branch Creek, which was treated with antimycin on 4 October 1972, and control station MD on untreated Maple Dale Creek. The triangles near SB1 and MD indicate flood control reservoirs.

Fork of the Kickapoo River. A 5-ha permanent impoundment on the stream, 4 km above its mouth, serves as a flood control reservoir.

Because Seas Branch Creek was treated with antimycin at its source, a nearby stream, Maple Dale Creek, which has similar physical, chemical, and biological characteristics, was selected as a control. This stream, 4 km long, is a tributary of Bishop Branch Creek, which is also a tributary of the West Fork of the Kickapoo River. The confluence of Bishop Branch Creek with the Kickapoo is 9 km downstream from that of Seas Branch Creek. A flood control structure, with no permanent pool, is about 300 m above the sampling station on Maple Dale Creek.

The upstream Seas Branch Creek station (SB1) was 50 m above the impoundment, in the middle of a riffle 70 m long. At this station the stream averaged 1.5 m in width and 12 cm in depth, and had a mean annual discharge of $0.11 \text{ m}^3/\text{s}$. The downstream Seas Branch station (SB2) was 800 m below the impoundment and was at the lower end of a riffle 130 m long.

The stream here was 3.3 m wide and 18 cm deep, and had a mean annual discharge of $0.17 \text{ m}^3/\text{s}$. The Maple Dale Creek control station (MD) was 6 m above the confluence of Maple Dale and Cook Creeks at the lower end of a riffle 40 m long. The stream here was 2.5 m wide and 15 cm deep, and had a mean annual discharge of $0.15 \text{ m}^3/\text{s}$.

Water quality data were taken at each station throughout the study period (Tables 1, 2, and 3). Temperatures ranged from 0 to 20 C; average temperatures at SB1 were 2 to 4 C lower than at SB2 or MD, or both, during the summer before and the two summers after treatment. Water chemistry differed little at the stations before, during, or after treatment. Dissolved oxygen concentrations were high (8.5 to 15.1 mg/l; 81 to 126% saturation). The calcareous composition of the numerous bluffs along both streams is reflected in the average water quality values: pH, 8.3; total alkalinity, 215 mg/l; and conductivity, 434 μmhos . Turbidity was low, averaging 0.29 Jackson Turbidity Unit (JTU) during periods of normal flow (Tables 1, 2, and 3). Average discharge at all stations was about twice as high in 1973 as in 1972 or 1974. Slow release of cold groundwater after high precipitation in 1973 may have been responsible for the lower water temperatures in 1973 and 1974.

The stream bed at all three stations was composed largely of rough, angulate stones, mostly 5 to 10 cm in diameter (some up to 30 cm). Small amounts of gravel and sand were present; interstitial organic litter was primarily autochthonous plant material. The stones at SB2 and MD were loose, but at SB1 many were imbedded in clay. Water crowfoot (*Ranunculus aquatilis*), the dominant stream vegetation, covered 8 to 50% of the stream bed throughout the year at all three stations. Limited amounts of pondweed (*Potamogeton* sp.) were present in the control stream, and watercress (*Nasturtium officinale*) along the water's edge in the treatment stream.

Methods

Antimycin (Fintrol-concentrate formulation) was applied to Seas Branch Creek from 0000 to 0920 h on 4 October 1972, under the direction of the Cold Water Research Group of the Wisconsin Department of Natural Resources. Errors in calculating dosages and equipment failure resulted in treatment values much higher than the intended concentrations of $10 \mu\text{g/l}$. The concentration was $25 \mu\text{g/l}$ for the first 3 h and $40 \mu\text{g/l}$ for the next 6-1/3 h at SB1 and $17 \mu\text{g/l}$ for 2 h and $44 \mu\text{g/l}$ for 7 h at SB2. Antimycin drip sites were about 270 m above each of the two stations.

Table 1. *Physical and chemical data from the upper treatment station (SB1) of Seas Branch Creek, 1972-74.*

Date	Temp (°C)		Dissolved O ₂		pH	Total alkalinity (ppm)	Turbidity (JTU)	Conductance (μmho/cm)	Discharge (m ³ /s)
	Air	Water	ppm	Percent saturation					
1972									
15 May	21	16	11.7	122	8.8	186	0.16	ND	0.08
15 June	15	12	10.1	96	8.5	194	0.27	440	0.08
15 July	32	17	9.2	98	8.1	186	0.12	440	0.08
15 Aug	25	14	10.0	100	8.5	203	0.30	420	0.07
3 Oct	18	11	10.1	95	8.3	215	0.27	520	0.09
6 Oct	10	10	10.2	94	8.4	223	0.57	500	0.08
13 Oct	10	10	11.0	102	8.5	213	0.36	480	0.08
1 Nov	8	8	10.6	93	8.3	215	0.19	420	0.10
1 Dec	-6	4	13.0	102	8.2	207	0.35	400	0.08
1973									
15 Jan	-12	1	14.3	104	8.3	203	0.30	400	0.08
15 March	1	6	13.4	111	8.2	210	0.21	380	0.15
15 May	15	8	12.8	112	8.6	218	0.11	450	0.17
16 July	24	14	11.4	115	8.3	203	0.22	460	0.19
15 Sept	10	9	9.9	90	8.0	198	0.27	440	0.16
6 Oct	11	9	10.3	95	8.1	236	0.17	450	0.14
10 Oct	1	5	12.4	100	8.1	209	0.20	410	0.13
1974									
20 April	10	9	10.2	90	8.1	210	0.20	350	0.11
17 May	16	11	10.4	97	8.2	215	0.50	420	0.04
30 July	21	14	9.8	98	8.0	233	0.07	400	0.09
2 Oct	6	9	10.0	90	7.4	207	0.16	410	0.10
Mean	11.8	9.9	11.0	100	8.2	209	0.25	430	0.11

On 16 August 1972 the gate of the impoundment on Seas Branch Creek was opened and the reservoir drained to an area of 1 ha, where it was maintained until after treatment to reduce the amount of antimycin needed. When the gate was closed 2 days after treatment, the impoundment refilled in about 2 weeks. This refilling reduced the water flow at SB2 by about one-half for most of that period.

Benthic samples were collected a total of 20 times during the 28-month study. On each sampling date, four samples were taken at SB1 and five each at SB2 and MD with a modified Hess circular 0.05-m² bottom sampler (with a net of 7.5 meshes/cm), similar to that described by Waters and Knapp (1961). At each station one sample came from vegetation and the rest from the rubble substrate. The biomass of benthos in the vegetation samples was prorated into the total benthic biomass according to the estimated percentage of the riffle area covered with vegetation at each sampling period. This percentage was assigned subjectively on the basis of the estimated change in

vegetative cover in each riffle area from one sampling period to another.

Drifting organisms were collected in vertical nets of 7.5 meshes/cm supported by a 0.1-m² square frame attached to a board placed on the stream bottom. Three nets were used at SB2 and MD, and two at SB1 to collect samples for 10 min, four or five times in each 24-h period before treatment. Sampling times included sunrise, sunset, midday, and midnight, which represented times of major drift (Waters 1972). Drift samples were taken every 3 h for 24 h during treatment (starting 3 h before application of antimycin) and then every 6 h for 36 h thereafter. Total drift was calculated by the methods of Waters (1962). Current velocities were measured with a Gurley pigmy current meter, no. 625. Velocities were used to calculate discharge, which was then used to calculate drift rates.

Samples of invertebrates were strained with a 0.5-mm mesh soil screen and stored in 70% isopropyl alcohol. Organisms were separated from detritus and

Table 2. *Physical and chemical data from the lower treatment station (SB2) of Seas Branch Creek, 1972-74.*

Date	Temp (°C)		Dissolved O ₂		pH	Total alkalinity (ppm)	Turbidity (JTU)	Conductance (μmho/cm)	Discharge (m ³ /s)
	Air	Water	ppm	Percent saturation					
1972									
15 May	18	14	11.7	115	8.7	186	0.11	ND	0.10
15 June	17	15	9.8	100	8.6	199	0.16	420	0.11
15 July	32	20	10.0	114	8.0	190	0.73	420	0.12
15 Aug	24	18	11.6	125	8.5	201	0.44	400	0.10
3 Oct	17	12	10.3	98	8.3	220	0.52	500	0.16
6 Oct	10	11	10.8	102	8.5	220	0.30	500	0.15
13 Oct	10	10	13.0	119	8.5	240	0.42	520	0.05
1 Nov	8	8	11.6	102	8.5	203	0.20	440	0.20
1 Dec	-6	4	12.5	100	8.1	215	0.19	420	0.15
1973									
15 Jan	-12	1	14.3	103	8.0	224	0.40	460	0.12
15 March	1	6	14.1	117	8.3	185	7.20	320	0.22
15 May	15	12	13.1	125	8.5	197	0.58	425	0.24
16 July	24	17	11.1	118	8.1	204	0.51	420	0.26
15 Sept	10	12	9.1	85	8.0	220	0.42	420	0.29
6 Oct	11	11	10.0	95	8.1	214	0.21	440	0.31
10 Nov	0	4	12.2	98	8.0	214	0.31	420	0.28
1974									
20 April	10	9	9.6	85	7.8	202	0.16	350	0.18
17 May	16	12	10.3	100	8.1	217	0.20	400	0.06
30 July	20	17	8.9	95	8.2	220	0.10	410	0.08
2 Oct	7	10	10.4	95	7.7	232	0.35	400	0.16
Mean	12.1	11.2	11.2	105	8.3	210	0.35	425	0.17

identified, and body length was measured. Identifications were verified by the museum staff of the Smithsonian Institution, Washington, D.C. In estimating the biomass of individual organisms from the length, we followed Hynes (1961), Hynes and Coleman (1968), Hamilton (1969), and Jacobi (1969) in assuming that an insect's shape is that of a cylinder five times as long as wide, that its volume increases by the cube of the length, that its specific gravity is 1.05, and that 3.3×10^{-5} g is the weight of a 1-mm length unit. Insects were not weighed because weight loss varies widely after preservation (Howmiller 1972). Crayfish were wet-weighted after surface water had been removed by blotting.

Additional specimens from some of the major taxa were nonquantitatively collected from the treatment and control stream on 6, 13, and 19 October and 4 November 1972, and the percentages of dead organisms noted (Table 4). We used these values to estimate the percentages of dead organisms in the benthic samples for these periods; biomass was

adjusted to show only the weight of living organisms. The taxa collected are given in Table 5; average monthly values for water temperature, discharge, vegetative cover, and total benthic biomass before and after treatment in Table 6; and the estimated biomass (g/m²) for each organism at each station on each collection date in Tables 7-9.

Results

Total Benthos and Drift

The aquatic macroinvertebrates collected included 33 identified to genus or genus and species, 5 to family, and 2 to order (Table 5). The dominant forms on the basis of pretreatment biomass (in order of abundance) were *Hydropsyche* (caddis fly), *Orconectes propinquus* (crayfish), Chironomidae (midges), *Optioservus fastiditus* (riffle beetle), *Anotocha* (crane fly), *Brachycentrus americanus* (caddis fly), *Gammarus pseudolimnaeus* (scud),

Table 3. *Physical and chemical data from the control station (MD) of Maple Dale Creek.*

Date	Temp (°C)		Dissolved O ₂		pH	Total alkalinity (ppm)	Turbidity (JTU)	Conductance (μ mho/cm)	Discharge (m ³ /s)
	Air	Water	ppm	Percent saturation					
1972									
15 May	18	18	8.5	93	8.6	191	0.40	ND	0.09
15 June	15	13	11.6	113	8.6	216	0.23	420	0.08
15 July	28	20	10.2	116	8.6	219	0.23	470	0.10
15 Aug	28	20	11.0	124	8.5	213	0.13	420	0.07
3 Oct	16	13	10.1	98	8.4	238	ND	ND	0.13
6 Oct	13	11	8.6	81	8.5	235	0.47	560	0.11
13 Oct	6	10	11.0	101	8.5	226	0.30	540	0.09
1 Nov	9	8	10.9	95	8.5	222	0.60	450	0.15
1 Dec	-6	4	13.8	107	8.3	230	0.10	440	0.11
1973									
15 Jan	-11	0	15.1	106	8.2	223	0.30	500	0.10
15 March	1	5	14.5	117	8.4	229	0.21	420	0.33
15 May	15	15	12.4	126	8.7	210	0.21	440	0.25
16 July	24	19	9.9	105	8.1	238	0.55	460	0.16
15 Sept	15	10	10.4	95	8.1	228	0.30	450	0.20
6 Oct	11	10	11.8	108	8.1	206	0.17	480	0.17
10 Nov	1	5	13.1	105	8.1	230	0.19	440	0.17
1974									
20 April	16	13	10.1	98	8.4	224	0.10	345	0.17
17 May	18	13	11.2	110	8.3	223	0.35	420	0.06
30 July	21	17	9.7	102	8.3	247	0.10	425	0.12
2 Oct	7	7	12.3	105	7.6	248	0.20	385	0.10
Mean	12.3	11.6	11.3	105	8.3	225	0.27	447	0.14

Baetis cingulatus (mayfly), and *Prosimulium* sp. (black fly).

Drift rates increased noticeably during treatment at both stations, reaching 50 g/h at SB1 18 h after treatment and nearly 169 g/h at SB2 9 h after treatment (Fig. 2). Other increases in drift rates were associated with increased densities or emergence of the dominant taxa before and after treatment (Fig. 3). The high values for total drift at SB2 in July and October 1974 are attributed largely to scuds, which in these 2 months constituted 67% and 34% of the total benthic biomass.

Total benthic biomass decreased at SB1 and SB2 (as well as at MD) immediately after treatment but attained a peak in the treated stream later in the fall, resuming the generally increasing trend that began in early fall (Fig. 4). One year after treatment, total benthic biomass in Seas Branch Creek approached or exceeded that found before treatment. This trend also was suggested during the second year after treatment, although the order of dominating taxa differed between years.

The decrease in benthos at the control station (MD) during the time of treatment probably reflects a sampling error, rather than a true decrease in density of organisms; the samples were collected from a riffle area which had been disturbed during earlier sampling. Neither drift samples (Fig. 3) nor nonquantitative benthic samples (Table 4) indicated abnormally high values for dead or drifting organisms at MD during the time of treatment.

To compare the pretreatment and posttreatment data, we calculated the average biomass (without crayfish) of samples collected annually at each station in May, July, and October (Table 6). Biomass reached maximum levels during these months, which span the major growing season. Vegetative cover more than doubled during the year after treatment at SB2 and during the second year after treatment at SB1, but changed little at the control station. Benthic biomass also followed this general pattern in the treatment stream but, again, remained nearly constant in the control stream (Table 6).

Table 4. Summary of total numbers of invertebrates collected and percentage dead at the three study stations on different dates after the antimycin treatment on 4 October 1972. (Dashes indicate no sample taken; P = present, but not counted).

Date (1972) and taxon (L = larvae)	Station					
	SB1		SB2		MD	
	Total no.	Dead (%)	Total no.	Dead (%)	Total no.	Dead (%)
6 October ^a						
<i>Baetis</i>	77	13	13	85	32	0
<i>Brachycentrus</i>	47	53	31	74	100	1
<i>Gammarus</i>	49	37	19	100	27	4
<i>Hydropsyche</i>	51	12	6	33	48	0
<i>Optioservus</i> (L)	32	16	2	0	27	0
<i>Antocha</i> (L)	41	63	—	—	4	0
<i>Stenonema</i>	11	9	4	0	1	0
<i>Orconectes</i>	0	0	10	0	0	0
13 October						
<i>Baetis</i>	1	100	0	0	16	0
<i>Brachycentrus</i>	12	50	50	98	31	3
<i>Gammarus</i>	17	18	4	50	50	2
<i>Hydropsyche</i>	58	40	100	89	50	0
<i>Optioservus</i> (L)	33	15	35	20	25	0
<i>Antocha</i> (L)	7	43	8	100	25	12
<i>Stenonema</i>	4	25	6	83	4	0
<i>Orconectes</i>	3	0	4	0	3	0
19 October						
<i>Baetis</i>	9	0	0	0	28	0
<i>Brachycentrus</i>	5	40	20	100	55	2
<i>Gammarus</i>	9	0	3	0	69	2
<i>Hydropsyche</i>	19	58	7	100	55	2
<i>Optioservus</i> (L)	26	4	25	0	53	2
<i>Antocha</i> (L)	9	33	2	100	41	7
<i>Stenonema</i>	3	0	2	50	10	0
<i>Orconectes</i>	10	0	4	0	3	0
4 November						
<i>Baetis</i>	0	0	0	0	—	—
<i>Brachycentrus</i>	10	80	P	0	—	—
<i>Gammarus</i>	4	25	P	0	—	—
<i>Hydropsyche</i>	5	0	P	0	—	—
<i>Optioservus</i> (L)	2	0	P	0	—	—
<i>Antocha</i> (L)	2	50	P	0	—	—
<i>Stenonema</i>	2	0	P	0	—	—
<i>Orconectes</i>	0	0	P	0	—	—

^a Data for SB2 on 6 October were obtained from observations on organisms placed in small containers before treatment.

Amphipoda (Scuds)

Immediately after treatment, *Gammarus pseudolimnaeus* decreased in the benthic samples (Fig. 4), and increased markedly in the drift (Fig. 2). The number of drifting dead and dying organisms reached a maximum 12 h after treatment at SB2 and 18 h after treatment at SB1 (Fig. 2). By the second day after treatment the mortality of scuds was apparently 100% at SB2 but only 37% at SB1 (Table 4). Estimated benthic biomass of scuds at both treatment locations remained low during the winter after treatment but increased in the following summer to values far exceeding those of the previous year (Fig. 4). Scuds were abundant in the benthos during the summer after treatment; they were also dominant in July 1974 at both treatment stations, making up 56% and 67% of the biomass (without crayfish) at SB1 and SB2. A drift rate at SB2 of 5 g/h in September 1973 and about 25 g/h in July and 24 g/h in October 1974 reflected this increased density of organisms (Fig. 3).

In the control stream, the biomass of scuds never varied significantly from one sample period to another (Fig. 4), and drift rates were low throughout the year (Fig. 3).

Diptera (Crane fly, Midges, Black fly)

Benthic biomass of the crane fly *Antocha* was reduced sharply by the treatment at both SB1 and SB2 (Fig. 4), and no live specimens were collected in the drift immediately after treatment. The drift of dead crane flies reached a peak 18 h after treatment at SB1 and 12 h after treatment at SB2 (Fig. 2). No living crane fly larvae were taken in benthic samples for 2 weeks at SB2 (none were found 2 days after treatment), whereas the maximum mortality of 63% at SB1 2 days after treatment decreased gradually to 50% (one of two specimens collected) 1 month later (Table 4). Benthic biomass of crane flies was about four times greater at SB2 than at SB1 before treatment but remained low for 1 year after treatment at both stations. The estimated biomass was high in the samples collected at SB1 in November 1973, but was again low in April and May 1974. Emerging adults were not found at SB1 during May 1974, but were present in drift samples at SB2 and MD. Despite the large numbers of larval crane flies in the samples collected at SB1 in November 1973, the population did

Table 5. Macroinvertebrate taxa collected in the treatment stream, Seas Branch Creek, and the control stream, Maple Dale Creek.^a

Arthropoda	Odonata
Insecta	Zygoptera (damselflies)
Diptera	Hemiptera (bugs)
Chironomidae (midges)	Corixidae (water boatmen)
Tipulidae (crane flies)	<i>Sigaria mathesoni</i>
<i>Antocha</i>	Belostomatidae (giant water bug)
<i>Hexatoma</i>	<i>Lethocerus</i>
Simuliidae (black flies)	Gerridae (water striders)
<i>Prosimulium</i>	<i>Gerris</i>
Empididae (dance flies)	<i>Trepobates</i>
<i>Hermerodromia</i>	Crustacea
Rhagionidae (snipe flies)	Amphipoda
<i>Atherix variegata</i>	Gammaridae (scud)
Stratiomyidae (soldier flies)	<i>Gammarus pseudolimnaeus</i>
<i>Hedriodiscus</i>	Decapoda (crayfish)
Tabanidae (horseflies)	Astacidae
<i>Tabanus</i>	<i>Orconectes propinquus</i>
<i>Chrysops</i>	Arachnoidea
Ephemeroptera (mayflies)	Hydracarina (water mites)
Baetidae	Mollusca
<i>Baetis cingulatus</i>	Gastropoda (snails)
Heptageniidae	Basommatophora
<i>Stenonema</i>	Physidae
Ephemerellidae	<i>Physa obtrussoides</i>
<i>Ephemerella</i>	Pelecypoda (clams)
Trichoptera (caddis flies)	Heterodonta
Brachycentridae	Sphaeriidae
<i>Brachycentrus americanus</i>	<i>Pisidium</i>
Hydropsychidae	Annelida
<i>Hydropsyche</i>	Hirudinea (leeches)
<i>Cheumatopsyche</i>	Rhynchobdellida
Hydroptilidae	Glossiphoniidae
<i>Ochrotrichia</i>	<i>Glossiphonia complanata</i>
Helicopsychidae	Arhynchobdellida
<i>Helicopsyche borealis</i>	Erpobdellidae
Glossosomatidae	<i>Erpobdella punctata</i>
<i>Protophila</i>	Oligochaeta (worms)
<i>Glossosoma</i>	Pleisopora
Limnephilidae	Tubificidae
Plecoptera (stoneflies)	Platyhelminthes
Perlodidae	Turbellaria (flatworms)
<i>Isoperla</i>	Tricladida
Nemouridae	Planariidae
Coleoptera (beetles)	<i>Dugesia</i>
Elmidae (riffle beetles)	Nematomorpha
<i>Optioservus fastiditus</i>	Gordiida (horsehair worms)
<i>Stenelmis sandersoni</i>	Gordiidae
Dytiscidae (diving beetles)	<i>Gordius</i>

^a All forms shown were collected in both the treatment and control stream, with four exceptions: *Erpobdella*, *Helicopsyche*, and *Nemoura* were only in the treatment stream and *Pisidium* only in the control stream.

Table 6. Average monthly (May, July, and October^a) water temperature, discharge, vegetative cover, and benthic biomass at Seas Branch Creek stations SB1 and SB2 and control station MD before (1972) and after (1973, 1974) the antimycin treatment of Seas Branch Creek.

Station and year	Water temp (°C)	Discharge (m ³ /s)	Estimated vegetative cover (%)	Benthic biomass (g/m ²)
SB1				
1972	15	0.08	10	56.5
1973	10	0.17	10	49.7
1974	11	0.08	23	114.8
SB2				
1972	15	0.13	11	61.1
1973	13	0.27	27	105.6
1974	13	0.10	17	111.0
MD				
1972	17	0.11	11	96.0
1973	15	0.19	9	93.2
1974	12	0.11	7	100.3

^a Before treatment on 3 October 1972 for all stations.

not recover from the treatment—as indicated by the sharp (nearly complete) overwinter decline, the lack of adults in the succeeding summer drift, and the near absence of the organisms in October 1974 (2 years after treatment).

The rate of emergence of crane flies was high in spring and decreased from May through September at the control station; the sharp decrease in biomass between March and May 1974 (Fig. 4) was presumably a result of emergence. Larval drift rates were low throughout the year at both treatment stations, except for the increase at the time of treatment (Fig. 3).

Drift rates of Chironomidae at SB2 increased sharply 21 h after treatment, peaked 12 h later, then declined gradually into the next week; drift at SB1 increased slightly 15 h after treatment (Fig. 2). The biomass at both SB1 and SB2 decreased slightly during treatment, then increased sharply in December 1972 (Fig. 5). At this time, midges dominated the biomass (without crayfish) at both treatment stations, contributing 57% at SB1 and 63% at SB2. Apparently the larvae rapidly occupied habitats vacated by more sensitive organisms. Biomass then decreased throughout the year to low levels that approached pretreatment values. High drift rates of midges at SB2 in the year after treatment (Fig. 3) were attributed to overlapping hatches of the various species present.

Benthic biomass values of Chironomidae were low and fluctuated throughout the year at station MD; an increase in biomass similar to that at SB1 and SB2 occurred here after the treatment date, but never reached the levels found at the treated stations (Fig. 5). Drift rates were low throughout the year at MD; the nearly 5 g/h in May 1974 (Fig. 3) reflected the slightly higher benthic biomass present then (Fig. 5).

Antimycin had no direct effect on *Prosimulium* sp. because black flies had emerged before treatment. Biomass at SB2 remained low through November; an increase began in January that reached a maximum of 98 g/m² in July 1973 (Fig. 5), or 65% of the benthic biomass (without crayfish). Drift rates, which previously were low (not illustrated) increased with this large increase of *Prosimulium*. Biomass at SB1 also peaked in July. A residual population was present at MD, throughout the year, but never made up a significant portion of the total benthic biomass (Fig. 5).

The dance fly *Hemerodromia* sp. which was present at all three stations in small numbers (but ranging up to 8 g/m² at station MD in January 1973) throughout the year (Tables 7–9) appeared to be unaffected by the treatment; few specimens were in the drift, and no dead ones were found.

Ephemeroptera (Mayflies)

Many dead nymphs of *Baetis cingulatus* were observed at the time of treatment at SB1, and drift rates doubled (Fig. 6). At SB2, where the water was warmer, a major emergence had taken place before the treatment. Benthic biomass of this species therefore declined at both stations after treatment (Fig. 5). The benthic biomass of *B. cingulatus* increased 20-fold 1 year after treatment at SB2 and also increased greatly at SB1 earlier in the year (Fig. 5). The decrease in biomass at all stations in November 1973 (Fig. 5) was apparently caused largely by earlier increased drift of late instar nymphs and subimagos (Fig. 7). The very high biomass levels at SB2 during the second summer after treatment were related to increased vegetation and the larger population of the generation in the preceding year. The decrease in biomass in October 1974 at SB1 was presumably due to earlier emergence.

The two periods of maximum emergence of *B. cingulatus* at MD were in May to July and late September to November. Benthic biomass increased here for each generation (Fig. 5), and drift was high at the time of emergence, which coincided with the time of antimycin treatment (Figs. 6 and 7).

The mayfly *Stenonema* sp. was present at the three sampling stations throughout the study; biomass was highest in the second year after treatment.

Mortality during treatment appeared to be initially low or nil at SB2, but five of six organisms (83%) collected 10 days after treatment were dead (Table 4).

Another mayfly, *Ephemerella* sp., was not collected in 1972 or 1973 but appeared in 1974 at SB1 in April, May, and October and at SB2 and MD in May.

Trichoptera (Caddis flies)

Benthic biomass of the caddis fly *Brachycentrus americanus* was reduced immediately after treatment (Fig. 8), and drift increased sharply (Figs. 6 and 7). At SB1, drift did not occur until 12 h after treatment and reached a maximum 3 h later (Fig. 6). Mortality at this station was about 53% 2 days after treatment and 80% 1 month later (Table 4). Drift at SB2 doubled shortly after treatment (Fig. 6) and continued to be high for at least 2 days. Mortality was 74% on the second day after treatment and 100% 2 weeks after treatment (Table 4). This species seemed to become disoriented during the antimycin treatment. At SB2 many organisms moved about sluggishly and crawled onto stream vegetation and stones. About 50% then abandoned their cases and died.

Biomass of *B. americanus* remained low at both treatment stations during the first year after treatment, but increased considerably at SB1 (not at SB2) during the second year after treatment (Fig. 8).

This species overwintered as larvae and emerged in May through August in Maple Dale Creek. An early emergence in May 1973 preceded a rapid increase in biomass of the following generation (Fig. 8).

For the caddis fly *Hydropsyche* sp., the number of dead and dying in the drift at SB2 reached a maximum 9 h after treatment and decreased during the next week (Fig. 6). Mortality then increased gradually to 100% on 19 October (Table 4). Few drift organisms were taken after treatment at SB1, and the number increased only slowly into the next week (Fig. 6). Mortality here was initially low and reached a maximum of only 58% 2 weeks later (Table 4). Biomass of *Hydropsyche* was reduced at both stations during treatment and increased slightly during the months after treatment (Fig. 8). We attributed this increase to recolonization by drift. The population appeared to have recovered during the year after treatment. Biomass levels at both treatment stations during the second summer after treatment exceeded those before treatment.

In 1972, benthic biomass of *Hydropsyche* was markedly lower in samples taken at MD on 6 and 13 October than on 3 October or 1 November (Fig. 8). However, as mentioned earlier, this decrease was attributed to a sampling error as no increase in drift rates or die-off was observed during this period. Larvae in the 3 October and 1 November samples

were in the same size group, indicating that no emergence had occurred.

Hydropsyche produced one generation per year; emergence extended from May through August. Drift rates increased at the times of emergence (Fig. 7). Benthic biomass at all three stations showed decreasing trends after emergence, followed by an increase as the new generation developed (Fig. 8). Samples contained a wide range of instars because of the prolonged hatching time, as was observed also by Hynes (1961). Biomass increased in the fall. Medium size specimens (5-10 mm long) dominated the October-November samples and large ones (10-13 mm long) the late winter and early spring collections.

Coleoptera (Riffle beetle)

No noticeable changes in benthic biomass or drift of *Optioservus fastiditus* occurred during treatment, although benthos collections 10 days after treatment suggested a 15% and 20% mortality at stations SB1 and SB2, respectively (Table 4). The biomass of *O. fastiditus*, represented by concurrent populations of larvae and adults, reached a peak at all stations at about the same time in 1972 and 1973 (Fig. 8). After the reduction or disappearance of organisms sensitive to antimycin, the larvae contributed significantly to the total benthic biomass—e.g., up to 70% at SB2 on 13 October 1972 (Table 8).

Decapoda (Crayfish)

No dead or dying *Orconectes propinquus* were observed during or after the antimycin treatment (Table 4). Because this organism is highly mobile, it was difficult to accurately evaluate its population density (Tables 7, 8, and 9). Many young of the year (1.2-2.0 cm long) were found at SB2 from May, June, or July through October in all years (Table 8).

Discussion

The application of high concentrations of antimycin (17-44 $\mu\text{g/l}$) resulted in an immediate increase in drift rates and a temporary reduction in populations of five of nine major taxa, *Gammarus pseudolimnaeus*, *Antocha*, *Baetis cingulatus*, *Brachycentrus americanus*, and *Hydropsyche*. *Orconectes propinquus* was not affected by the treatment. The biomass of other organisms, such as Chironomidae, *Optioservus fastiditus*, and *Prosimulium*, increased during the months after treatment. Total benthic biomass (all taxa combined) during the two summers after treatment approached or exceeded that of the summer before treatment.

Table 7. *Benthic biomass (g/m²) for station SB1 of Seas Branch Creek above the impoundment, before and after treatment of the stream with antimycin on October 4, 1972.^a*

Taxon	1972								
	15 May	15 June	15 July	15 Aug	3 Oct	6 Oct	13 Oct	1 Nov	1 Dec
Diptera									
Chironomidae	11.5	4.0	8.1	2.3	2.2	T	0.4	6.7	102.1
<i>Antocha</i>									
larvae	4.3	1.7	4.6	2.3	3.2	0.9	0.2	0.1	0.3
pupae	2.9	0.8	0.5	0.4	0	0	0	0	0
<i>Prosimulium</i>									
larvae	T	0.3	0.7	0.5	0.1	0	0	0	0
pupae	T	0	T	T	T	T	0	0	0
<i>Hemerodromia</i>	0	0	0.2	0.1	3.7	1.2	1.0	1.1	1.5
Other	0.1	0.2	3.6	2.8	7.1	0.5	1.3	4.0	40.0
Ephemeroptera									
<i>Baetis</i>	0.1	1.0	0.9	2.1	5.2	0.3	0	0	0
<i>Stenonema</i>	0	0	0	0.1	0.6	0.9	0	0.2	0.5
<i>Ephemerella</i>	0	0	0	0	0	0	0	0	0
Trichoptera									
<i>Hydropsyche</i>	17.4	17.0	7.0	11.7	54.7	28.3	12.6	9.0	16.9
<i>Brachycentrus americanus</i>	0	0.4	2.8	1.5	1.6	0.4	0.5	0.1	1.3
<i>Ochrotrichia</i>	0.3	0.3	0	0	0	0	0	0	0
<i>Glossosoma</i>	0	0	0	0	0	0	0	0	0
Other	0.2	0.2	0.6	0.1	0.1	0.5	0.2	T	0.1
Plecoptera									
<i>Isoperla</i>	T	0	0	0	T	T	0	0	0.2
Coleoptera									
<i>Optioservus fastiditus</i>									
larvae	1.1	2.4	3.9	7.4	13.4	5.3	7.0	12.7	15.8
adult	0.3	0.1	0.3	1.0	0.9	0.5	0.5	0.3	0.7
<i>Stenelmis sandersoni</i>	0.1	0.1	0.2	0.2	0.7	0.1	0.2	0.3	0.1
Amphipoda									
<i>Gammarus pseudolimnaeus</i>	0.6	6.9	1.9	2.8	1.3	0.4	0.4	0.4	0.1
Mollusca									
<i>Physa ohrussoides</i>	0	0	0	0	0	0	0	0.1	0
Hirudinea									
<i>Erpobdella punctata</i>	0	0	0	0.3	0	0.9	0	T	0
Miscellaneous	0.3	T	0.2	0.1	0.1	0	0	T	T
Benthic Biomass									
without <i>Orconectes</i>	39.2	35.4	35.5	35.7	94.9	40.2	24.3	35.0	179.6
Decapoda									
<i>Orconectes propinquus</i>	4.2	0.1	16.7	11.9	0	1.4	0	0	4.0
Total Benthic Biomass	43.4	35.5	52.2	47.6	94.9	41.6	24.3	35.0	183.6

^a T = less than 0.05 g.

Table 7—Continued

1973							1974			
15 Jan	15 Mar	15 May	16 July	15 Sept	6 Oct	10 Nov	20 April	17 May	30 July	2 Oct
23.7	9.9	9.8	11.8	7.1	0.2	0.1	1.1	0.5	8.8	0.1
0.3	0.2	0.1	0.1	1.6	0.4	7.5	0.3	0.2	0.7	0.1
0	0	0	0	0	0	0	0	0	0	0
0.2	0.1	0	24.5	3.5	2.1	10.3	T	T	8.9	0.6
0	0	T	0	T	T	0	0	0	0	0
3.9	0.1	T	0.2	2.2	0.2	0.4	0	0	T	3.2
14.8	15.2	4.8	0	0.5	0	9.0	0	0.5	0	4.0
T	3.9	6.3	1.3	1.6	1.4	1.2	0.3	T	2.5	0.1
0.1	0	1.0	0	0.4	0.4	0.6	0.5	2.9	2.4	0.9
0	0	0	0	0	0	0	4.8	11.4	0	T
25.8	19.1	1.7	0.4	44.8	58.2	13.1	49.7	68.8	17.8	35.5
0.8	0.6	0	1.6	1.4	2.3	11.0	3.0	0	14.3	15.4
0	0	T	0	0	T	0	0	0	T	T
0	0	0	T	0	T	0	3.3	4.7	3.1	4.0
0.4	0.3	1.6	0.2	2.0	0.2	0	1.9	4.5	0.1	T
T	0.7	0.9	0	T	T	0.3	2.1	0.2	0	0
12.2	1.6	1.4	0.8	16.4	6.6	20.1	4.6	6.3	6.6	9.1
1.0	0.2	0.3	0.2	1.8	0.6	0.4	1.0	0.9	1.3	1.1
0.1	0	0	0	0.1	0	0.2	0	T	0	0
0.2	0.2	1.6	0.6	5.6	4.3	9.2	5.2	7.6	86.4	11.2
0.3	0	0	T	T	T	0	T	T	0	0.1
T	0	0	T	3.4	0.4	0.4	0	0.6	0.5	0
T	0	0.3	0	0.1	T	T	0	0	0	0
83.8	52.1	29.8	41.7	92.5	77.3	83.8	77.8	109.1	153.4	85.4
3.6	0	0	0	0	25.9	0	0	39.1	13.2	82.6
87.4	52.1	29.8	41.7	92.5	103.2	83.8	77.8	148.2	166.6	168.0

Table 8. *Benthic biomass (g/m²) for station SB2 of Seas Branch Creek, below the impoundment, before and after treatment of the stream with antimycin on October 4, 1972.^a*

Taxon	1972								
	15 May	15 June	15 July	15 Aug	3 Oct	6 Oct	13 Oct	1 Nov	1 Dec
Diptera									
Chironomidae	13.8	31.0	15.4	28.6	0.8	2.3	5.2	20.0	111.4
<i>Antocha</i>									
larvae	3.4	1.3	2.9	16.1	12.3	6.7	0	0.3	0.3
pupae	0.5	0.9	0.2	0.4	0	0.1	0	0	0
<i>Prosimulium</i>									
larvae	0	0.5	0.2	T	0.2	0	0	0	0.1
pupae	0	0.1	T	T	T	T	T	0	0
<i>Hemerodromia</i>	0	T	0.5	0	0.3	3.0	0.1	1.2	1.2
Other	0.6	0.2	T	0.2	0	0.4	0.1	16.3	1.8
Ephemeroptera									
<i>Baetis</i>	0.1	1.3	1.5	0.4	T	0	0	0	0
<i>Stenonema</i>	0	0	0	0.1	0.2	0.1	0.1	0.2	0.3
<i>Ephemerella</i>	0	0	0	0	0	0	0	0	0
Trichoptera									
<i>Hydropsyche</i>	6.7	12.1	17.4	30.1	34.2	19.7	4.1	7.2	10.4
<i>Brachycentrus americanus</i>	0	12.4	12.4	11.0	17.0	11.5	0.9	1.6	2.6
<i>Ochrotrichia</i>	T	0.1	0	0.1	0	0	0	0	0
<i>Glossosoma</i>	0	0	0	0	0	0	0	0	0
Other	1.1	0.6	0.3	T	T	0.1	0.3	0.5	0.2
Plecoptera									
<i>Isoperla</i>	0	0	0	0	0	0	0	0	0
Coleoptera									
<i>Optioservus fastiditus</i>									
larvae	0.4	5.4	8.6	7.3	12.3	17.7	40.5	45.7	43.3
adult	T	0.1	T	0.1	0.1	0.1	0.3	0.2	0.2
<i>Stenelmis sandersoni</i>	T	0.1	0.5	0.5	0.6	0.5	1.2	0.5	0.8
Amphipoda									
<i>Gammarus pseudolimnaeus</i>	4.9	4.9	1.7	2.4	2.3	0	0.1	T	0
Mollusca									
<i>Physa ohrussoides</i>	0	0.1	0	T	0	0	T	T	0.4
Hirudinea									
<i>Erpobdella punctata</i>	4.6	13.0	0	3.5	3.5	0	4.3	0.4	0.1
Nematomorpha	0	0	0	0	T	T	0	0	T
Miscellaneous	1.8	0.4	T	0	0.1	0.1	0.1	0.3	3.1
Benthic Biomass									
without <i>Orconectes</i>	37.9	84.5	61.6	100.8	83.9	62.3	57.3	94.4	176.2
Decapoda									
<i>Orconectes propinquus</i>	23.3	6.0	7.1	34.0	52.7	21.4	5.3	49.1	0
Total Benthic Biomass	61.2	90.5	68.7	134.8	136.6	83.7	62.6	143.5	176.2

^a T = less than 0.05 g.

Table 8—Continued

1973							1974			
15 Jan	15 Mar	15 May	16 July	15 Sept	6 Oct	10 Nov	20 April	17 May	30 July	2 Oct
50.5	39.3	55.3	13.9	8.8	0.1	0.1	0.6	3.2	0.4	0.1
0.1	T	0.5	0.1	0.6	0.5	0.5	0.3	1.1	0.4	1.5
0	0	0	0	T	0	0	0	0	0	0
0.7	3.4	1.9	97.8	0.4	0.1	0.1	T	3.1	1.3	T
0	0	0.1	T	T	T	0	0	0	0	0
2.9	T	0	T	2.2	0.6	1.1	0	0	T	0
3.6	11.0	0.3	0	0.4	0.2	0	1.3	0.4	1.2	9.2
T	0.9	3.4	6.1	13.9	14.0	1.3	23.5	37.7	6.0	7.6
0.1	0.1	0.1	T	1.5	2.6	2.3	8.9	8.4	1.1	1.4
0	0	0	0	0	0	0	0	1.5	0	0
4.7	4.5	3.8	2.5	39.1	39.1	11.9	10.9	50.9	6.6	18.7
0.3	0.5	0	1.1	1.2	0.5	0.3	0.1	0	2.4	3.3
0	0	0	T	T	T	0	0	0	0	0
0	0	0	T	0	0	0	0	0.3	0	0.3
3.0	1.6	0	T	0	0	9.0	0	0.1	0.3	0
0	0	T	0	T	0	0	1.1	0.2	0	0
18.5	9.4	1.5	10.5	33.2	18.9	20.6	7.3	8.2	10.2	14.3
0.1	0.2	0.2	0.2	0.3	0.1	T	0.2	0.4	0.6	0.8
0.2	0.1	T	T	0.1	T	T	T	0.3	0	0
0	T	0.1	11.6	24.0	16.3	14.5	19.7	20.8	71.4	29.3
0.4	T	T	0.2	0.1	T	T	T	0	0.3	0.2
0.1	2.8	20.6	1.0	0.9	1.0	1.6	12.7	2.9	5.1	T
0	0	0	0	0	0	0	0	0	0	0
2.0	0.7	0.4	0	0	T	0	0	0	0	0
87.2	74.5	88.2	145.0	126.7	94.0	63.3	86.6	139.5	107.3	86.7
12.6	8.9	5.3	25.0	45.2	80.7	15.4	15.3	16.7	27.9	13.1
99.8	83.4	93.5	170.0	171.9	174.7	78.7	101.9	156.2	135.2	99.8

Table 9. *Benthic biomass (g/m²) for control station MD of Maple Dale Creek, before and after treatment of Seas Branch Creek with antimycin on October 4, 1972.^a*

Taxon	1972								
	15 May	15 June	15 July	15 Aug	3 Oct	6 Oct	13 Oct	1 Nov	1 Dec
Diptera									
Chironomidae	5.0	10.7	3.6	6.3	1.0	0.7	1.6	2.0	21.9
Antocha									
larvae	1.6	0.4	12.7	10.9	11.9	13.0	7.1	13.1	7.9
pupae	0.7	0.2	0.3	1.8	0	0	0	0	0
Prosimulium									
larvae	0	0	0.2	0.3	0.1	T	T	0.2	0.2
pupae	0	0	T	T	T	T	0	0	0
Atherix	0	0	0	6.3	4.9	2.3	2.1	2.0	8.3
Hemerodromia	0.4	0.2	0	0.4	1.1	0.9	0.5	2.2	3.4
Other	0	0.7	T	T	0.1	0.2	0.9	0.1	T
Ephemeroptera									
Baetis	T	0.2	0.4	0.2	0.7	0.5	0.1	0.2	0.5
Stenonema	0.4	0	0	T	0.3	T	T	0.2	0.1
Ephemerella	0	0	0	0	0	0	0	0	0
Trichoptera									
Hydropsyche	9.8	6.3	14.7	60.3	172.9	40.2	6.0	164.9	40.3
Brachycentrus americanus	0.1	1.0	1.8	3.3	6.9	6.1	3.9	13.1	2.8
Ochrotrichia	0.1	T	0	0	0	0	0	0	0
Glossosoma	0	0	0	0	0	0	0	0	0
Other	0.8	0.3	T	0.1	0.6	0.4	0.6	0.1	0.1
Plecoptera									
Isoperla	0.3	0	0	0	T	T	0	0.2	0.4
Coleoptera									
Optioservus fastiditus									
larvae	1.4	4.0	4.6	4.2	21.2	13.6	17.8	19.7	11.4
adult	0.1	0.2	0.2	0.4	1.1	0.4	0.2	0.1	0.1
Stenelmis sandersoni	0.1	0.5	0.3	0.4	0.5	0.8	0.6	0.4	0.2
Amphipoda									
Gammarus pseudolimnaeus	0.7	2.3	0.8	0.4	0.5	0.1	0.5	0.2	1.3
Mollusca									
Physa ohrussoides	0.3	0.2	T	0	T	0	0	0.1	0
Hirudinea									
Erpobdella punctata	0	7.7	2.6	0	0.1	0	0	0	0
Nematomorpha	0	0	0	0	0	0	T	T	0
Miscellaneous	0.1	0.4	0.1	0.1	0.2	T	T	0.1	0
Benthic Biomass									
without Orconectes	21.9	35.3	42.3	95.4	224.1	79.2	41.9	218.9	98.9
Decapoda									
Orconectes propinquus	26.3	32.4	7.2	41.6	30.0	0.2	1.1	0	16.0
Total Benthic Biomass	48.2	67.7	49.5	137.0	254.1	79.4	43.0	218.9	114.9

^a T = less than 0.05 g.

Continued

Table 9. *Benthic biomass (g/m²) for control station MD of Maple Dale Creek, before and after treatment of Seas Branch Creek with antimycin on October 4, 1972.^a*

1973							1974			
15 Jan	15 Mar	15 May	16 July	15 Sept	6 Oct	10 Nov	20 April	17 May	30 July	2 Oct
16.8	18.6	5.6	1.6	2.7	0.8	0.2	11.4	24.6	9.4	0.1
7.1	14.9	4.3	5.9	8.8	9.9	6.1	6.6	6.5	13.3	2.1
0	0	0	0	T	0	0	0	0	0	0
0.3	T	0	0.3	1.5	0.4	T	0.4	0.4	2.6	0.2
0	0	0	0	0	0	0	0	0.3	0	0
1.6	2.0	0	0	1.3	0	0	0	0	0	0
8.0	2.0	0.1	0.3	1.5	2.6	1.6	0	T	0	0
0.4	3.4	0.2	0.6	0	0	0	0.4	0	0	0
0.4	5.6	0.5	1.3	3.6	3.3	0.4	0.1	0.3	3.7	7.3
T	0.4	T	0	0.1	T	T	0	1.1	0.1	0
0	0	0	0	0	0	0	0	T	0	0
44.4	82.0	18.5	14.1	79.7	128.2	56.5	62.3	32.4	13.7	94.3
10.4	9.5	0	11.1	32.9	25.6	31.9	3.4	0	2.5	42.5
0	0	0	0	0	0	0	0	0	0	0
0	0	0	T	0	0	0	0.1	0.1	T	0.1
T	1.7	0.3	0.1	0	T	0	0.1	0	0.4	0
0.9	0.4	0.3	0	0	T	0	1.5	0.4	0	0
29.9	2.6	1.5	16.3	50.6	20.7	27.0	9.3	7.9	12.4	17.1
0.1	0.3	0.2	1.0	0.4	1.2	0.4	0.6	0.9	0.5	0.5
0.6	0.1	0	T	T	T	T	0.1	0.3	0.2	0
0.6	1.9	0	1.0	1.6	1.4	2.0	0.3	0.3	1.4	1.0
T	T	0	0.1	T	T	0	0	T	T	0
0	0.8	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0.1	0.1	0	0	T	T	0	0	0	0	0
121.6	146.3	31.5	53.7	184.7	194.1	126.1	96.6	75.5	60.2	165.2
1.2	14.7	0	8.8	42.7	22.8	0	6.6	22.6	128.0	1.0
122.8	161.0	31.5	62.5	227.4	216.9	126.1	103.2	98.1	188.2	166.2

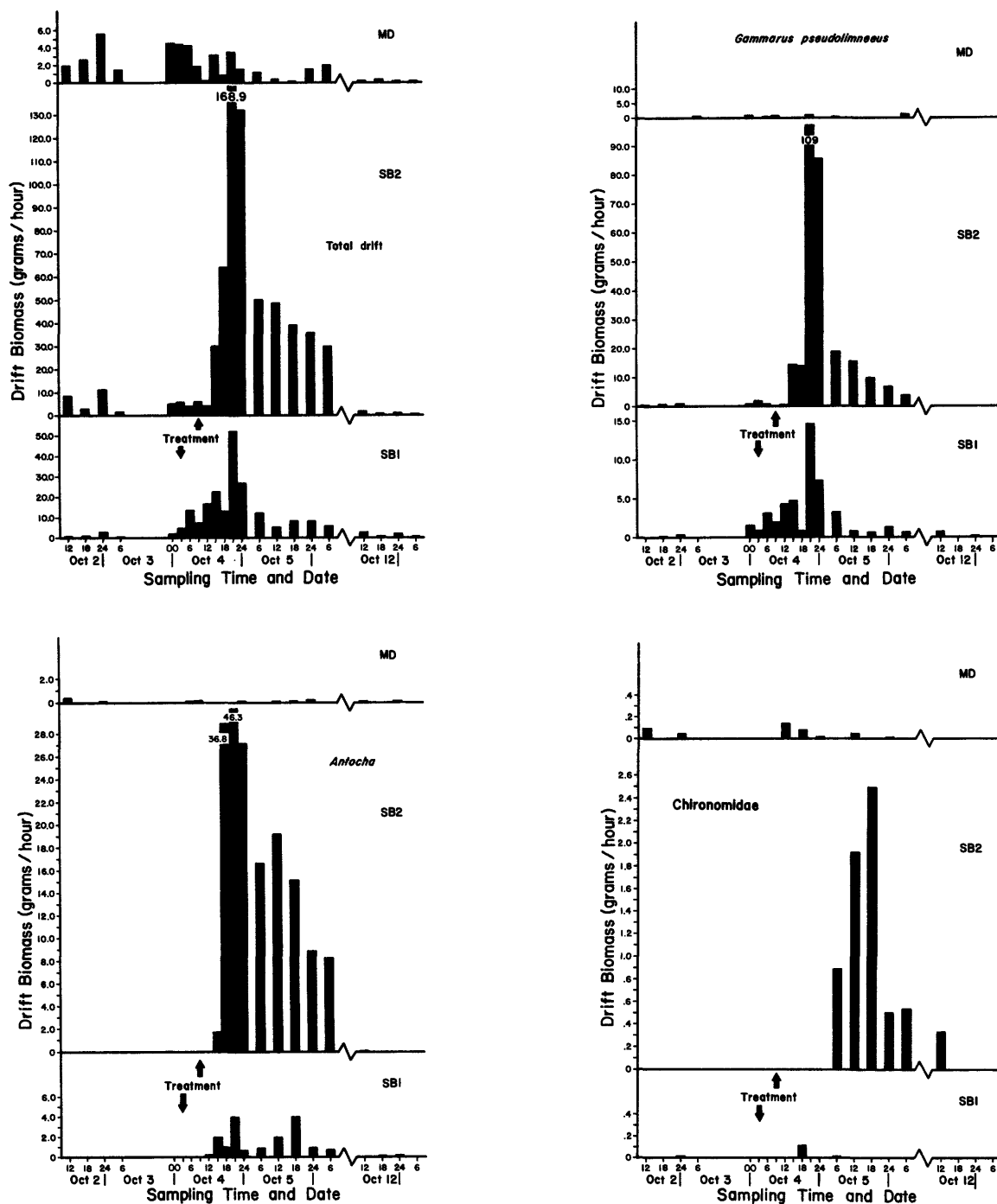


Fig. 2. Drift of benthic macroinvertebrates (total, scuds, crane flies, and midges) at sampling stations in treated Seas Branch Creek (SB1, above impoundment; SB2, below impoundment), and in untreated Maple Dale Creek (MD), October 1972. Numbers along the baseline show sampling times (6 = 0600 h, 12 = 1200 h, . . .) and arrows show time when antimycin reached the station.

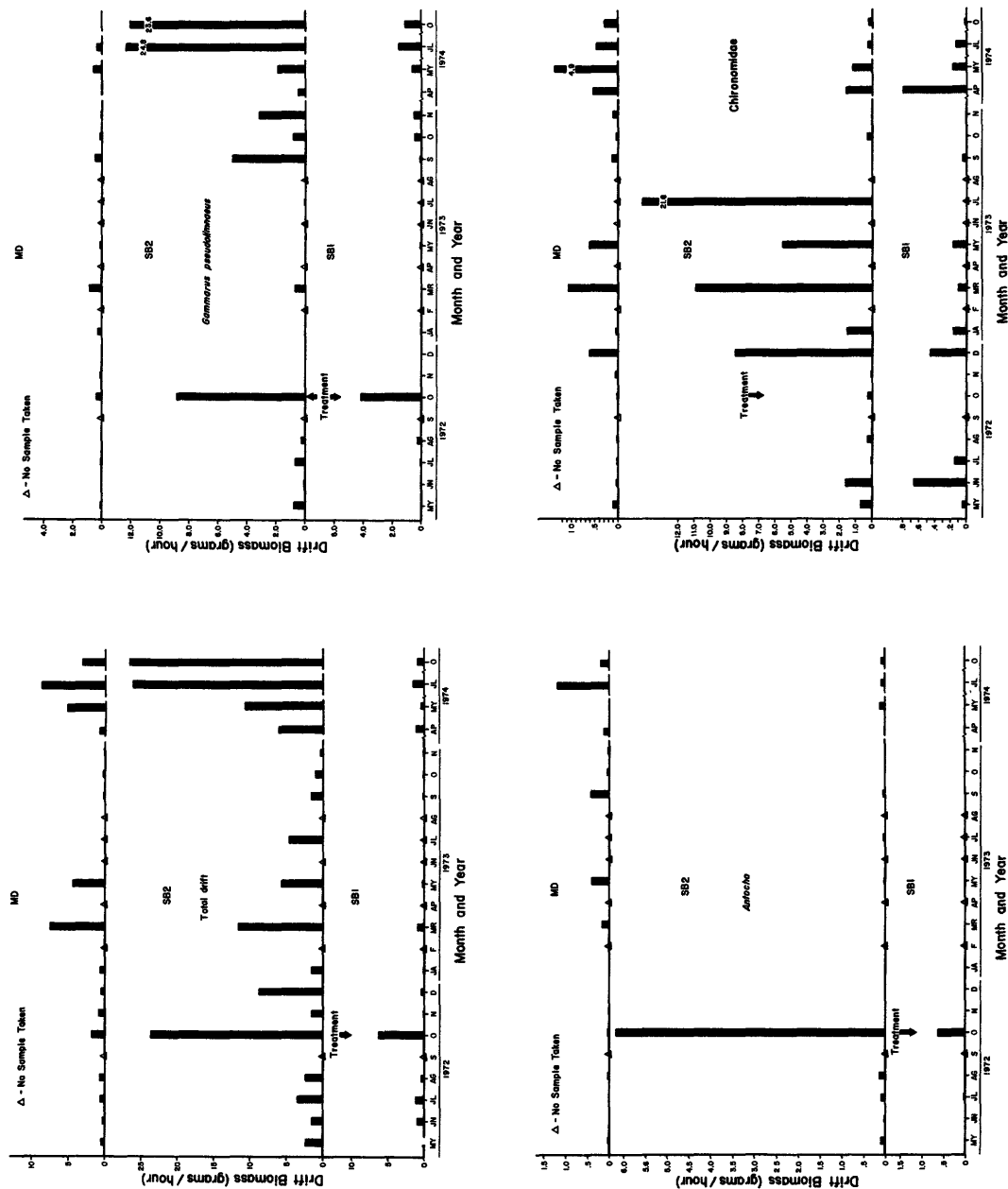


Fig. 3. Drift of benthic macroinvertebrates (total, scuds, crane flies, and midges) in treated Seas Branch Creek (SB1, SB2), and in untreated Maple Dale Creek (MD), 1972-74.

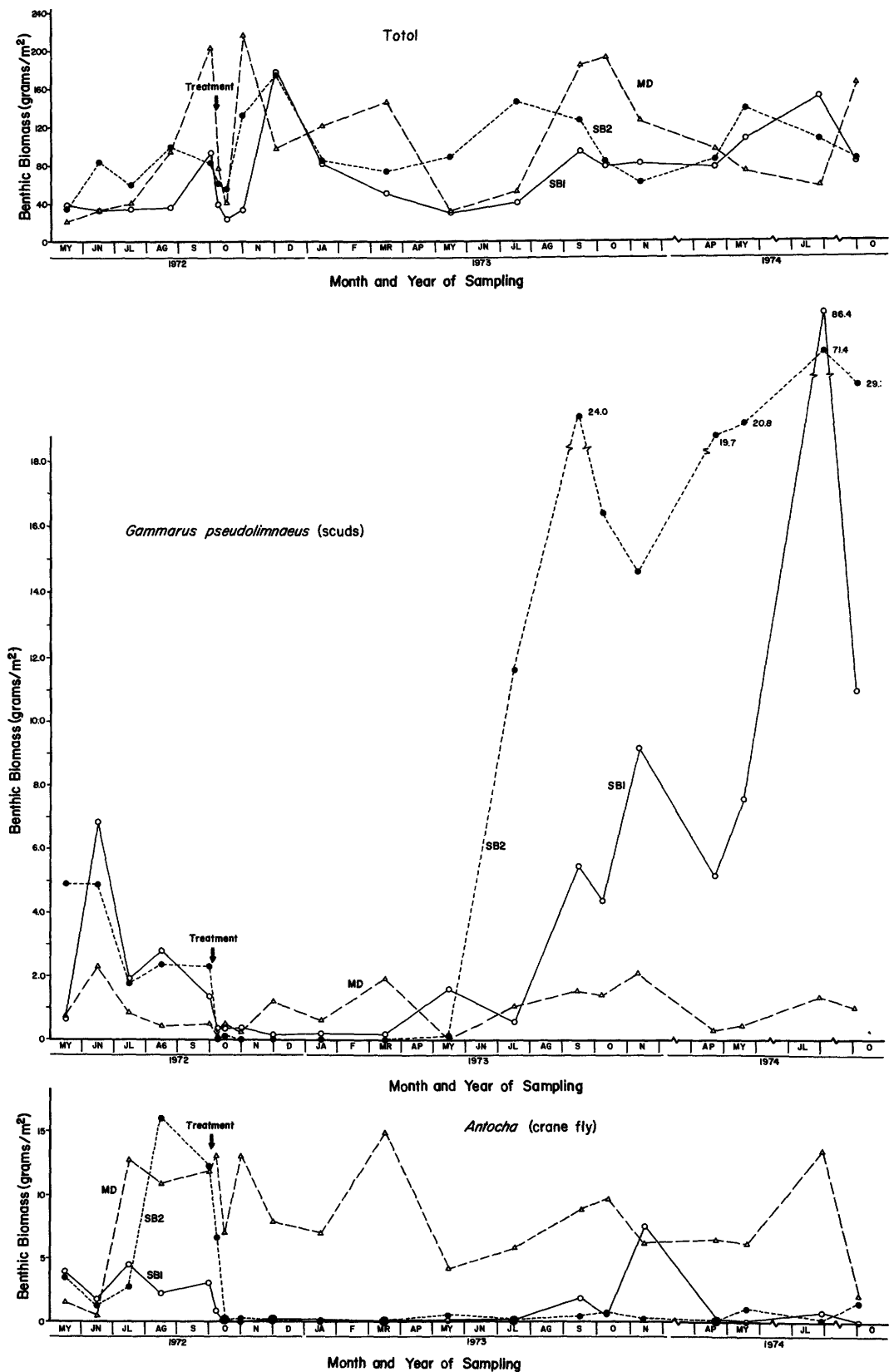


Fig. 4. Biomass of benthic macroinvertebrates (total, scuds, and crane flies) in treated Seas Branch Creek (SB1, SB2), and in untreated Maple Dale Creek (MD), 1972-74.

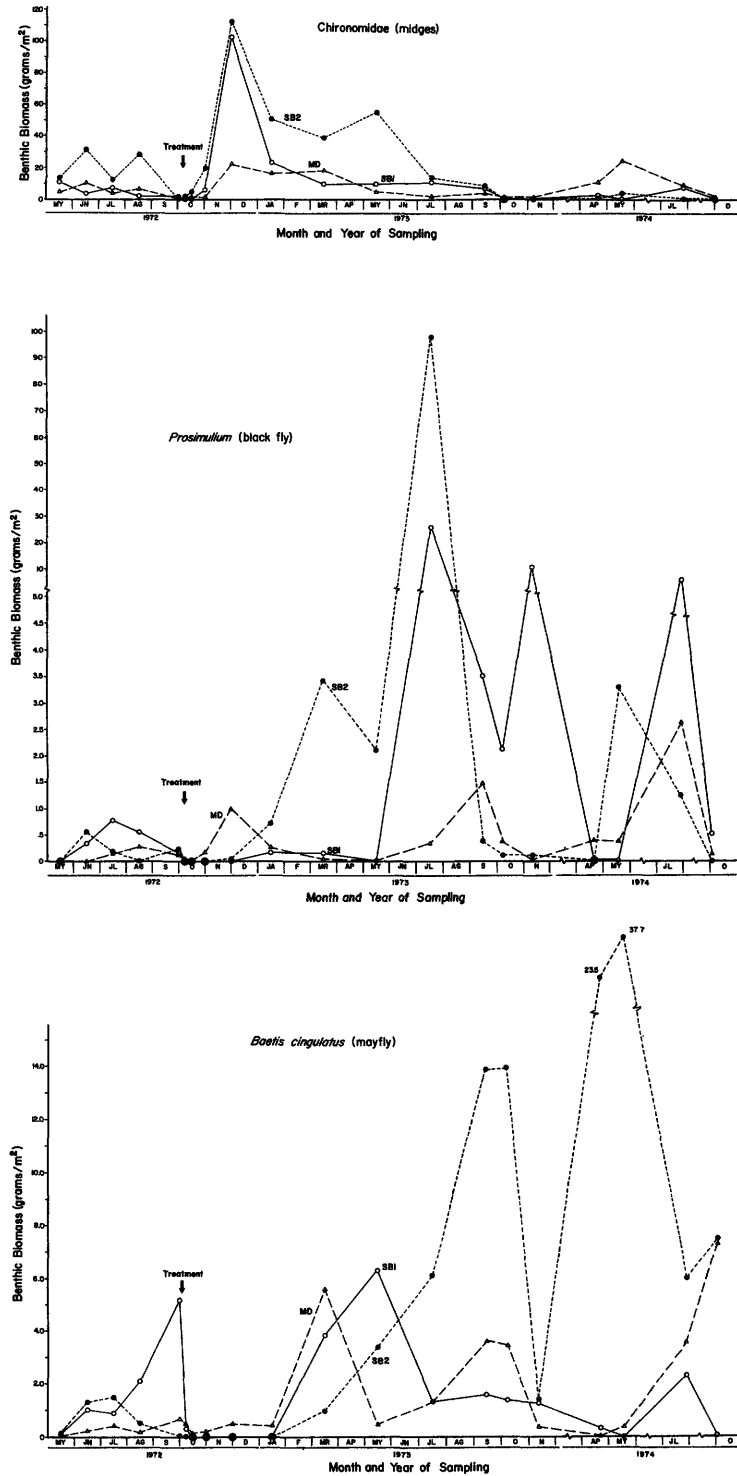


Fig. 5. Biomass of benthic macroinvertebrates (midges, black flies, and mayflies) in treated Seas Branch Creek (SB1, SB2), and in untreated Maple Dale Creek (MD), 1972-74. (Note the change in scale for biomass of black flies for values larger than 5 g/m²).

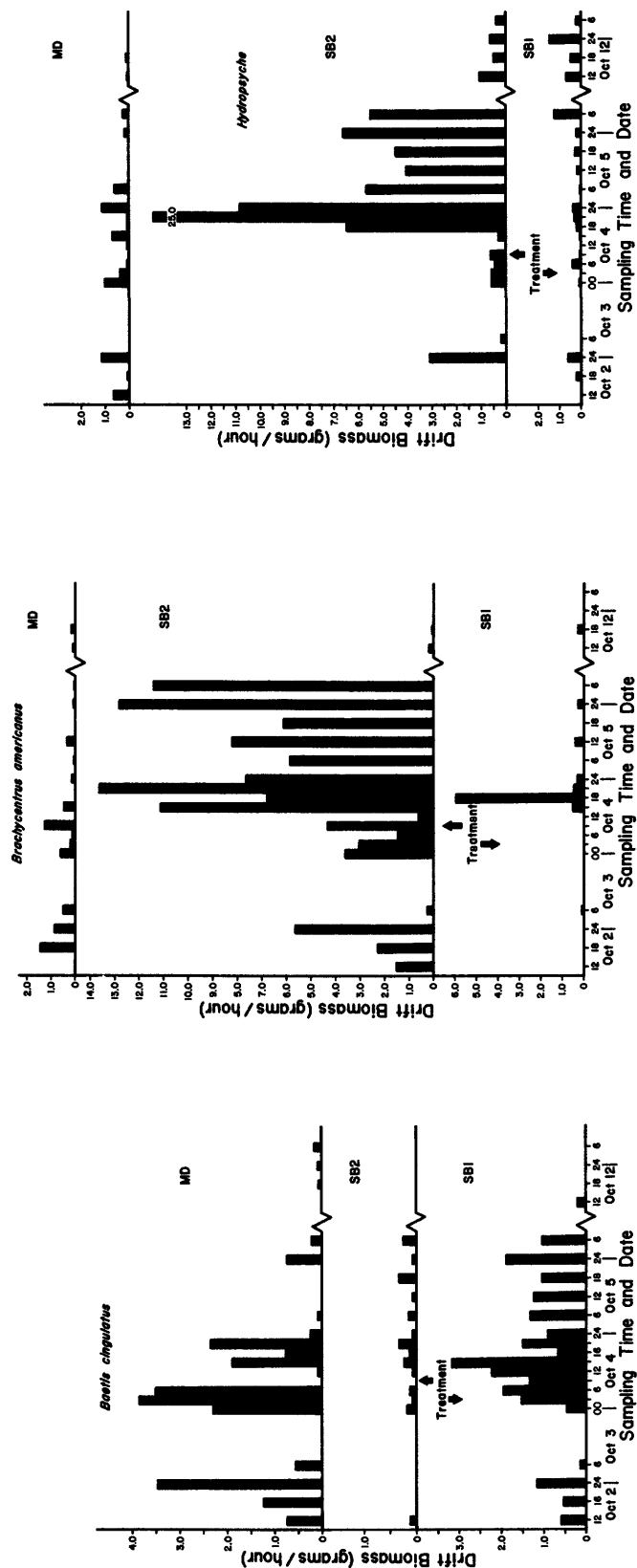


Fig. 6. Drift of benthic macroinvertebrates (a mayfly and two caddis flies) in treated Seas Branch Creek (SB1, SB2), and in untreated Maple Dale Creek (MD), October 1972.

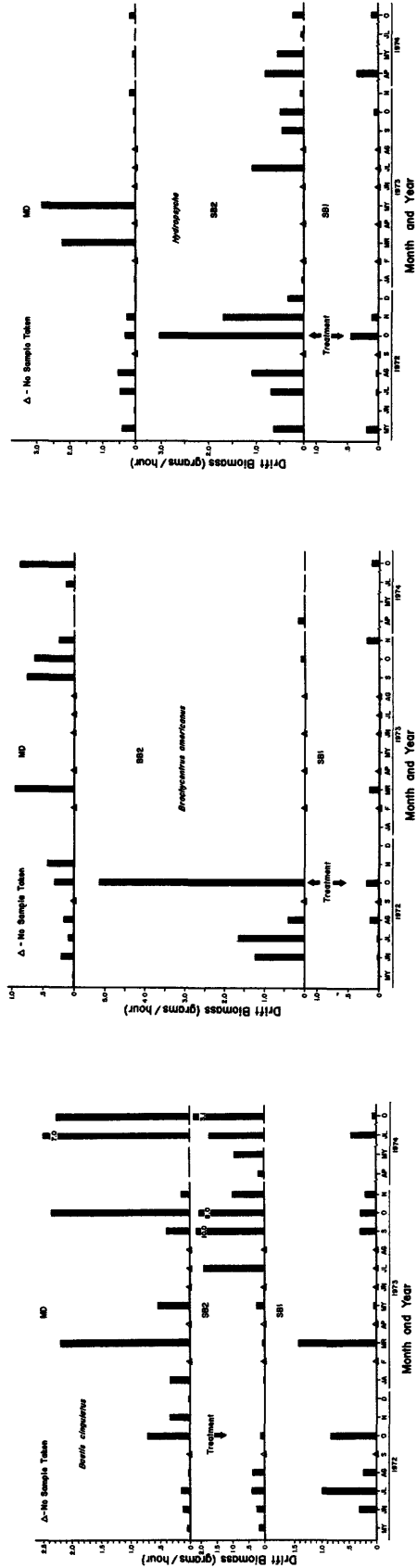


Fig. 7. Drift of benthic macroinvertebrates (a mayfly and two caddis flies) in treated Seas Branch Creek (SB1, SB2), and in untreated Maple Date Creek (MD), 1972-74.

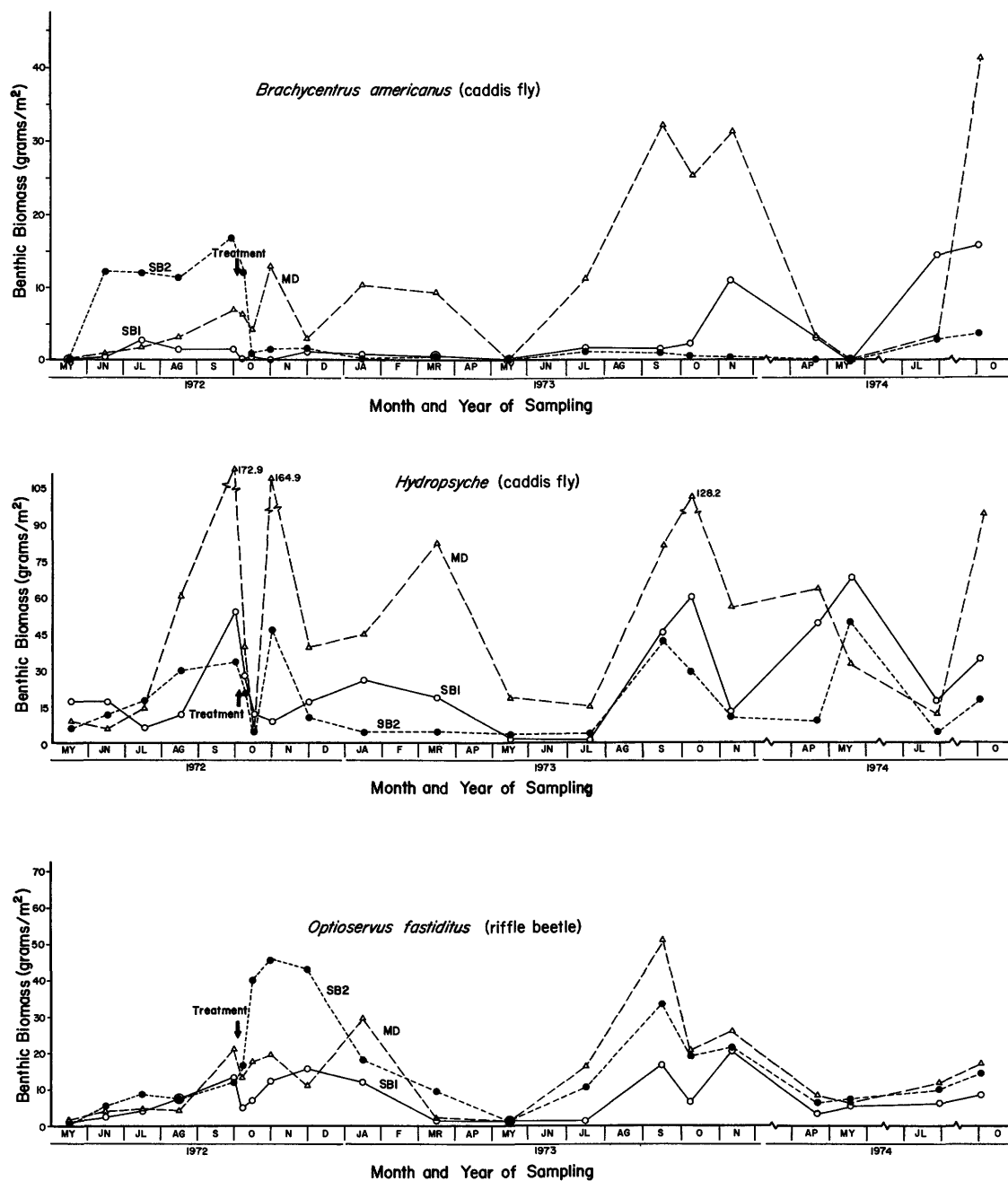


Fig. 8. Biomass of benthic macroinvertebrates (two caddis flies and a riffle beetle) in treated Seas Branch Creek (SB1, SB2), and in untreated Maple Dale Creek (MD), 1972-74.

Many benthic organisms are not specialized in food preference, and diets change according to the availability of algae (Chapman and Demory 1963). Additional food and space for Chironomidae, *O. fastidius*, and *Prosimulium* could result from the reduction of other taxa of invertebrates, and of fish, and an increase in algae and in available plant surface area for attachment. The alga, *Vaucheria* sp., increased noticeably at SB2 1 week after treatment and reappeared in June 1973. *Ranunculus* present in July increased here also from a maximum of 15% stream-bed coverage before treatment to 50% in the year after treatment.

Recovery of invertebrates after treatment may have been hastened by the increase in stream vegetation. Particulate organic matter flushed downstream when the reservoir was draining may have been a source of nutrients. Nutrients also may have been made available by bacterial and fungal degradation of fish carcasses which littered the stream bottom after treatment. An increase in nutrients was observed by Richey et al. (1975) when kokanee salmon (*Oncorhynchus nerka*) died after spawning.

Chironomidae, *Gammarus pseudolimnaeus*, *Baetis cingulatus*, and *Prosimulium*, which had high turnover rates resulting from immature developmental periods of less than 1 year, returned more quickly than most other taxa to pretreatment biomass levels in the year following treatment. Populations of *Antocha* and *Brachycentrus americanus*, which have longer development times, had not recovered to pretreatment levels 1 year after treatment at the downstream station (SB2). Moffett (1936) observed a similar pattern in populations that were decimated by floods. Although both *Antocha* and *B. americanus* showed signs of recovery in November 1973, *Antocha* dropped back to low levels at SB1 during the second year.

Hildebrand (1971), who studied benthos disruptions by salmon spawning, believed that organisms with low drift rates in winter would not repopulate a stream until midsummer, when drift rates increased. Rapid recolonization in Seas Branch Creek could have taken place because treatment of areas adjacent to the mainstream was incomplete; e.g., mortality of *Hydropsyche* was high at SB2 but recovery was rapid after treatment. Repopulation could also have resulted from the insects' normal recolonization cycle which Mueller (1954) found to involve upstream flight of adults, ovoposition, population growth, and a later downstream drift of immatures in response to competition for food and space.

Because antimycin is short-lived, it would be desirable, although somewhat difficult, to treat a stream when adults of dominant or sensitive insects

are mating. If ovoposition took place after treatment, survival and perhaps higher biomass levels might result, as observed in *Baetis cingulatus* at the downstream Seas Branch station.

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